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ABSTRACT BOOK

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Session 1,1

Type XVII collagen restoration via prime editing in junctional epidermolysis bullosa

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Introduction: Epidermolysis bullosa (EB) is a monogenetic disease, characterized by formation of extended blisters and lesions on the skin and mucous membranes upon minimal mechanical trauma. It is caused by pathogenic genetic variants of genes encoding proteins that are essential for skin integrity. Functional impairment, reduction, or absence of type XVII collagen (C17), due to genetic COL17A1 variants, is characterized by blistering within the lamina lucida of the basement membrane, resulting in junctional EB (JEB).

Methods: Here, we present a prime editing (PE)-based gene repair strategy for disease-associated COL17A1 variants in JEB. Primary human JEB keratinocytes were treated with PE mRNAs via electroporation. Restored C17 expression was analysed via immunofluorescence microscopy, flow cytometry, and Western blot. On-target and potential off-target effects were examined via NGS. Furthermore, accurate expression and deposition of restored C17 was analysed in a patient-derived xenograft mouse model.

Results: Prime editing resulted in C17 restoration in up to 60% of treated cells, which was accurately shed from the cell surface. Remarkably, in engineered skin xenografts derived from a mosaic of unedited and edited cells, in which C17-positive cells represented only 55.9% of the input population, we observed that the COL17A1-corrected cells populated 93.5% of the basal keratinocyte layer 6 weeks after grafting. This effect is in line with C17's known role in anchoring hemidesmosomes to the basement membrane, preserving the structural integrity of the stem cell niche, and promoting the longevity of corrected cells.

Conclusion: Our data highlights the suitability of PE as a therapeutic gene editing tool for EB and other genodermatoses.

Investigating the mechanism of matrix production in a novel keloid model

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Keloids, pathological scars characterized by excessive fibrosis, pose considerable challenges due to their disfiguring appearance and the limited understanding of their underlying pathomechanisms. Recent research suggested that the Schwann cell – M2 macrophage interaction plays a key role in keloid development.

To study keloid formation in in vitro, we have developed a 3D model utilizing the self-secreted extracellular matrix (ECM) of dermal cells from keloids and compared these to healthy skin. ECM assembly was investigated using multiphoton microscopy with 2nd harmonics generation. ECM component changes were investigated by ELISA of the supernatants. We conducted 3D co-cultures of healthy fibroblasts and Schwann cells with differentially polarized macrophages to explore their interactions and contributions to keloid formation and progression.

Similar to scar tissue in vivo the ECM secreted by the dermal in vitro keloid models displayed a higher ECM fibre alignment. The secretion of the ECM components was increased in the keloid compared to healthy skin models. The co-culture of macrophages with Schwann cells showed little effects on macrophages polarization. RNA sequencing revealed that co-cultured with monocytes showed only minor effects on Schwann cells, while gene expression changed significantly when co-cultured with M1 or M2 polarized macrophages. Contrary, co-cultures of Schwann cell with M1 macrophage induced an inflammatory phenotype in Schwann cells, whereas co-culture with M2 macrophages led to the induction of genes involved in ECM-assembly, indicating that the macrophage phenotype influence Schwann cell function.

Our findings show that our innovative in vitro keloid model can recapitulate the changes in extracellular matrix observed in keloids in vivo, enabling further research on the pathogenesis as well as potential treatment testing. The interaction of Schwann cells with M2 macrophages might recapitulate the crosstalk leading to matrix formation increase in Schwann cells already proposed in vivo, enabling studying this process in more detail in vitro.

Session 1,3

Impact of glutamate metabolism and its inhibition on melanoma development and myeloid cells

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Tumor immunity is negatively regulated by metabolites in the tumor tissue. Metabolic reprogramming impacts the activation and maturation of dendritic cells (DC). We work on the transgenic melanoma mouse model tg(Grm1)EPv which spontaneously develops melanoma due to overexpression of the metabotropic glutamate receptor 1 (Grm1) in melanocytes. This aberrant glutamate metabolism drives tumor formation, but might also affect immune cell function. Thus, we study the metabolic changes in progressing tg(Grm1)EPv melanoma and their possible effects on DC and T cell responses.
We performed analyses of myeloid subsets in tumors and draining lymph nodes during tumor progression with multicolor flow cytometry. While cDC2 and macrophages decreased, neutrophils and monocytes increased in the lesions. An investigation of DC precursors in the bone marrow and in vitro DC differentiation assays showed no difference between tumor-bearing tg(Grm1)EPv and C57BL/6 mice.

Metabolic screening of the tg(Grm1)EPv mice at different disease stages with LC-MS technology revealed a shift towards glutamate/glutamine, a shift from Glucose/Pyruvate to Lactate during glycolysis and a decrease in ATP. This data is supported by RNA sequencing results showing an increased expression of enzymes involved in glutamate metabolism and glycolysis. These changes indicate a Warburg effect, disruption of the respiratory chain, and metabolic changes in the tumor microenvironment that are advantageous for the tumor cells and unfavorable for DC.

Current investigations focus on alterations caused by glutamate pathway inhibition in vitro and in vivo. We see a significant increase in cell death in Grm1-positive melanoma cells after treatment with Buthionine sulphoximine (BSO) while not having an effect in Grm1-negative control cells. This knowledge can drive the design of novel therapeutic strategies for cancer patients involving potential modification of tumor glutamate metabolism. Combination therapies with inhibitors of the glutamate pathway might improve response rates in cancer patients.

Session 2,1

Gene expression profiles and immune cell composition of generalized pustular psoriasis and acute generalized exanthematous pustulosis

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The clinical and histopathological features of generalized pustular psoriasis (GPP), a neutrophilic condition, overlap with those of acute generalized exanthematous pustulosis (AGEP), making a rapid and reliable distinction difficult. The aim of this study was to investigate the molecular differences between AGEP and GPP.

In a multicenter team effort, we collected lesional FFPE skin biopsy samples from patients with AGEP (n=40), GPP (n=30), palmoplantar pustulosis (PPP; n=9), plaque psoriasis (PSO; n=8), cutaneous adverse drug reactions (ADR; n=12) and healthy control skin (HCO; n=9), then performed bulk RNA sequencing and blinded histopathological analyses. The transcriptomes as well as the histopathologic features of AGEP and GPP largely overlapped, but showed marked differences to PPP, PSO, ADR and healthy skin. Analysis of immune response patterns showed that GPP samples had the highest expression of Th17- and neutrophil-related genes, while AGEP had higher expression of cytotoxicity-related genes. Furthermore, using CIBERSORTx, a computational deconvolution of RNA-Seq data, we found a higher number of neutrophils in GPP but a higher number of CD8+ T cells in AGEP samples. Transcriptome profile subgroup analysis revealed that drug-induced GPP and AGEP exhibited nearly identical results whereas immune response pattern and immune cell composition of AGEP slightly differed from that of non-drug-induced GPP. Both conditions showed dysregulation of genes related to neutrophil and IL-36 inflammatory signaling. Together, this data suggest that AGEP is a (first event) drug-induced variant of GPP and therefore has more cytotoxic features than GPP.

The data were obtained in collaboration with CEE GPP expert network members R. Čeović, J.-T. Maul, M. Marovt, V. Mateeva, B. Meier-Schiesser, D. Meyersburg, G. Ratzinger, A. Reich, L. Pavlovsky, K. Prillinger, J. Semakova and A. Szegedi, who provided clinical data, samples and other support.

Session 2,2

JAK-inhibition re-directs skin inflammation from tissue destruction to pathogen clearance

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The use of JAK inhibitors is becoming more widespread in dermatological practice as they have been approved for the treatment of a wide range of inflammatory skin diseases including atopic dermatitis, vitiligo and alopecia areata. However, we currently lack a detailed understanding of their effects on the diverse host cell types. Recently, we identified the JAK-STAT1 pathway as a key driver in the pathogenesis of scarring alopecia and showed that therapeutic JAK inhibition could successfully restore tissue homeostasis and halt hair follicle destruction in preclinical mouse models. The therapeutic benefit of JAK inhibition in scarring alopecia patients has also been reported by us and others in several recent case studies, demonstrating the translatability of our findings.

Our mouse model has a hair follicle-specific EGFR deficiency, which leads to a skin barrier breakdown, inflammation involving a diverse immune infiltrate, bacterial dysbiosis and eventually destruction of the hair follicle stem cells and hair loss. To characterize the impact of JAK inhibition on this multifaceted skin inflammation, we now performed single cell RNA-sequencing of wildtype and mutant mice during progressive hair loss that were treated with topical JAK1/2 inhibitor ruxolitinib or vehicle for two weeks.

Our approach revealed that the immune infiltrate consists of T cells and innate lymphoid cells with varied polarization states. Notably, JAK1/2 inhibition preferentially suppressed Type 1 and Type 2 immune responses and cell compartments, resulting in a shift towards a more Type 17-dominated immune landscape with enhanced antibacterial properties.

Taken together, our study indicates that JAK1/2 signaling orchestrates a defined arm of the immune system aimed at tissue destruction and that JAK1/2 inhibition re-directs rather than completely suppresses the immune system to enhance microbial clearance. These findings should pave the way for a more targeted use of this novel class of therapeutics.

Session 2,3

Defining the Immunoregulatory Function and Transcriptomic Profile of Multinucleated Giant Cells in Cutaneous Sarcoidosis

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Multinucleated giant cells (MGCs) are central to granulomas in cutaneous sarcoidosis, yet their immunomodulatory role remains largely unexplored. Recently, MGCs have been associated with favorable outcomes in cancers such as squamous cell carcinoma and are thought to modulate immune responses by interacting closely with surrounding immune cells, including T cells and macrophages. This suggests that MGCs in sarcoidosis may similarly influence granuloma stability, potentially impacting disease progression and patient outcomes. However, a comprehensive functional profile of MGCs within sarcoid granulomas is still lacking.

To investigate the role of MGCs in cutaneous sarcoidosis, we performed single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics on granulomatous lesions and non-lesional skin biopsies from sarcoidosis patients. Additionally, we developed in vitro assays with patient-derived cells to examine the responsiveness of monocyte-derived macrophages to granuloma-specific cytokines.

Our findings reveal that certain granuloma-associated T cell-derived cytokines promote macrophage multinucleation, contributing to the recruitment of additional immune cells and impacting granuloma formation. scRNA-seq of sarcoidosis patient skin biopsies uncovers an MGC-specific gene signature enriched in granuloma-associated macrophages, highlighting genes involved in macrophage activation, cytokine signaling, and immune cell recruitment. Using spatial transcriptomics, we further demonstrate that MGC-specific transcripts localize closely within regions of high immune cell density in granuloma cores, surrounded by macrophages and T cells, identifying this gene signature as a unique marker of cutaneous sarcoidosis.

These findings position MGCs as potential regulators of immune responses within sarcoidosis granulomas, closely interacting with T cells and macrophages. Our study supports the MGC gene signature as a diagnostic marker for sarcoid granulomas and suggests that targeting MGC-mediated pathways may provide novel therapeutic options for managing chronic granulomatous inflammation in sarcoidosis.

Session 3,1

Advanced Techniques for Detecting Senescent Cells in Dermatoporosis: A Non-Invasive Approach.

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Cellular senescence is a fundamental mechanism in mammalian aging, including humans. Somatic cells enter senescence after multiple divisions, becoming detectable in healthy individuals at advanced ages. Animal models, particularly mice, demonstrate that senescent cells accumulate with age, driving the aging process. Removing these cells in mice results in tissue rejuvenation. In humans, external stressors, particularly UV exposure, can accelerate this process in the skin leading to age-related skin conditions like dermatoporosis. Dermatoporosis (DP), also known as "skin atrophy," is characterized by extremely dry, thin, and fragile skin. In addition to losing its protective function, the skin of DP patients often suffers from hemangiomas and necroses, which are particularly distressing. Full symptoms of DP typically appear around the age of 70, with a prevalence of about 30% in this age group. Early clinical signs may be visible from around 50 years old, but no definitive prevention strategies exist. The exact causes are not entirely understood, but prolonged UV light exposure is a major trigger. Additionally, patients undergoing high-dose adjuvant steroid therapy during chemotherapy can develop DP at relatively young ages. The fragile nature of dermatoporotic skin complicates biopsy collection, prompting the development of non-invasive diagnostic techniques.

In this context, we will present preliminary results on a novel, non-invasive method for detecting senescent cells using a combination of Raman spectroscopy, Near-Infrared (NIR) imaging and Artificial Intelligence (AI). In this preliminary study, we subjected 2D cultures of fibroblasts to UVB irradiation and compared them to a control group. This approach trained the system to recognize senescent cells. Following this, we analyzed 3D skin equivalents, containing varying percentages of senescent fibroblasts to more accurately simulate the aging process of the skin. This method allows us to assess senescent cells in the skin and monitor their elimination when applying senolytic treatments in the future.

Session 3,2

Guarding the Gates: The hair canal prevents pathogenic dysbiosis of the skin

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Hair follicles play a crucial role in maintaining mammalian skin barriers and protecting against environmental stressors. While they provide a unique niche for commensal skin bacteria, uncontrolled microbial proliferation within hair follicles can lead to folliculitis and facilitate cutaneous infection dissemination. This phenomenon is particularly evident in cancer patients undergoing anti-EGFR therapy, who often develop papulopustular eruptions with concomitant Staphylococcus aureus superinfections.

This study elucidates the specific cell population responsible for the hair follicle's microbial gatekeeping function. Through analysis of single-cell datasets, characterization of EGFR-dependent transcriptional signatures, and targeted knockout experiments in genetically engineered mice, we identified the EGR2/K79-positive hair canal as a critical antimicrobial bastion. Our findings demonstrate that EGFR regulates the expression of antimicrobial peptides (AMPs), including beta-defensin1/6 and SPRR1a/4, within the hair canal via the ERK signaling pathway. Notably, EGFR expression in fully differentiated sebocytes was found to be dispensable for the hair follicle's homeostatic defense mechanism.

Furthermore, we established the translational relevance of these findings to human skin. AMP homologues were found to be concentrated in K79-expressing cells in human hair follicles, with their overexpression evident in EGFR-ERK-dominant psoriatic skin conditions. These results provide critical mechanistic insights into the hair follicle's microbial defense strategy and have direct therapeutic implications for addressing folliculitis associated with EGFR-inhibitor-based anti-cancer therapies.

Session 3,3

Human type 17 tissue-resident memory T cells are dysfunctional in acute cutaneous graft-versus-host disease

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Skin tissue-resident memory T cells (TRM) regulate homeostasis, but are also involved in inflammation, for example in graft-versus-host disease (GVHD), which can occur after hematopoietic stem cell transplantation (HSCT). Given the scarcity of comprehensive human studies, we aimed to explore TRM development, maintenance and involvement in tissue-specific disease mechanisms in human skin. To investigate the role of TRM in GVHD pathology, we studied patients undergoing allogeneic HSCT and with acute GVHD. While circulating host T cells are eradicated through myeloablative conditioning prior to HSCT, host skin TRM persist and coexist with donor T cells in the skin for years.

We hypothesized that comparing pre-existing host and developing donor TRM after transplantation could reveal key insights into TRM heterogeneity, longevity and turnover in human skin. Additionally, studying TRM in GVHD will uncover new pathways involved in TRM-mediated pathogenic signaling.

For this study, we collected longitudinal blood and skin samples of HSCT patients and untreated aGVHD patients. Using single-cell sequencing we distinguished host and donor genotypes based on genetic disparities and examined transcriptional and clonal dynamics of T cells over time. Clonal analysis of donor cells with shared TCRs in skin and blood identified effector T cells as the major source of de novo skin TRM. However, a specific type-17 polarized TRM subset was functionally impaired and decreased in GVHD. At the same time there was a shift towards IFN-γ production and type 1-mediated inflammation. Following GVHD resolution, the TRM17 subset was restored, indicating that active type 1 signaling during GVHD prevents its formation and maintenance. Overall, our findings suggest the presence of distinct TRM lineages in human skin, with independent developmental cues. These insights could have broader implications for understanding tissue-specific diseases that involve diverse TRM populations.

Session 3,4

Tissue-resident T cells in the Kaposi sarcoma tumor microenvironment

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Introduction: Kaposi sarcoma (KS), which occurs in people with human immunodeficiency virus (HIV) infection, is both an AIDS-defining disease and the most common neoplasm in people living with HIV (PLWH). Decreased immunosurveillance and chronic viral infection likely contribute to KS progression. However, it remains unclear how lack of local immunity leads to uncontrolled KSHV infection and/or KS progression, and how this can be therapeutically targeted. This study aims to examine the KS tumor microenvironment (TME) in PLWH and HIV-negative individuals.

Methods: Pre-treatment samples from cutaneous KS from HIV+ individuals with low nadir and cutaneous KS from HIV- individuals were recruited. We mapped the KS tumor microenvironment (TME) using immunofluorescence imaging of tissue-resident T cell (TRM) subsets and associated cytokines.

Results: We observed increased numbers of T cells in KS samples compared to healthy skin. In the TME of HIV+ patients, overall T cell populations were higher than in HIV- patients. In contrast, HIV- patients had significantly elevated CD4+ T cells within the TME and CD8+ T cells specifically in the tumor. HIV- patients exhibited increased numbers of TRM within the TME, particularly CD8+ TRMs and displayed high levels of CXCR3+ CD8+ TRM in both the TME and tumor.
Additionally, HIV- patients presented with elevated levels of IFN-γ-positive T cells and IFN-γ-positive TRMs in both the tumor and TME.

Conclusion: The findings reveal significant differences in immune responses between HIV+ and HIV- KS patients. HIV- showed increased CD4+ and CD8+ T cells, particularly TRMs, which are less abundant in HIV+ patients. The elevated levels of CXCR3+ CD8+ TRMs and IFN-γ-producing TRMs in HIV- patients suggest that these cells may play a key role in promoting antitumor effects. In contrast, HIV+ patients demonstrated reduced infiltration of these cell types, which may indicate a diminished immune response to tumor cells in this population.

Session 4,1

Immune programs in human dendritic cells upon immunization with Chlamydia trachomatis

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Introduction: Chlamydia trachomatis (Ct) is the leading cause of bacterial sexually transmitted infections worldwide. Most infections are asymptomatic, allowing them to remain undetected, and since natural immunity is not robust, reinfections are common. Low-grade chronic inflammation in untreated patients can result in severe long-term compilations, such as infertility. In mouse models, protective immunity is associated with IL-12 secretion, Th1 cell activation, and IFNγ production. Our study aims to assess the role of human dendritic cell (DC) subsets in Ct infection, focusing on their early involvement in anti-chlamydial immune response and understanding why most individuals fail to develop protective immunity.

Methods: We are using monocyte derived DCs (moDCs) and conducting in vitro Ct infections of cervix samples from hysterectomy donors. To investigate immune mechanisms in Ct-immunized DC, we performed flow cytometric analysis, bulk RNA sequencing, and functional in vitro migration experiments.

Results: In cervix-derived immune cells infected in vitro with Ct, we found CD14+CD11c+ DC as the main immune cell subset taking up Ct, located in the stroma near epithelial cells, which are the primary infection targets. Mechanistically, moDCs stimulated with Ct showed upregulation of co-stimulatory molecules and the migration marker CCR7. Notably, Ct-infected moDCs displayed an elongated shape with an adhesive phenotype, while LPS-treated moDCs remained non-adherent. Despite these differences, non-adhesive moDCs exhibited similar migration patterns toward a CCL19 gradient across all conditions.

Outlook: Our next steps will investigate whether the adhesive phenotype of Ct-treated moDC impacts their ability to migrate, potentially explaining the lack of long-term immunity if DCs cannot reach T cells in lymph nodes. Additionally, we plan to explore the mechanisms driving this morphological change and attempt to modulate the adhesive behavior of Ct stimulated DCs.

Session 4,2

Unraveling the relationship between regulatory T cells and hair follicle immune privilege

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Hair follicles have developed a relative immune privilege to protect their structure and their stem cell reservoir from immune-mediated destruction. This protective mechanism involves downregulation of antigen presentation, active anti-inflammatory checkpoints and the attraction of regulatory T cells, creating an immune-inhibitory microenvironment around the hair follicle. Breakdown of this immune-inhibitory state during microbial skin inflammation can lead to scarring alopecia.

Our previous research demonstrated that hair follicle-specific epidermal growth factor receptor (EGFR) disruption leads to cell-intrinsic hypersensitivity of the JAK-STAT1 pathway, compromising immune privilege and skin barrier function. We identified interferon gamma-expressing natural killer cells and CD8+ T cells as drivers of this chronic inflammatory cascade. Therapeutic topical blockade of JAK1/2 signaling restored immune privilege, activated remaining stem cells to promote new hair growth, and improved skin inflammation and barrier function in preclinical mouse models and patients with scarring alopecia.

Intriguingly, we observed an accumulation of regulatory T cells in the inflammatory infiltrate of our scarring alopecia mouse model compared to the wild-type situation. JAK inhibition, however, significantly reduced this population. Through analysis of their transcriptional profile, we now aim to unravel the intricate relationship between JAK inhibition and Treg function during the breakdown of the immune privilege in hair follicles. We aim to discover potential targeted therapies and develop more effective and sustainable treatment strategies for scarring alopecia.

Session 4,3

Mechanosensitive fibroblasts regulate tissue stiffness via LOX secretion in psoriasis

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Psoriasis, a chronic inflammatory skin condition, is marked by a thickened epidermis with elongated rete ridges and significant infiltration of immune cells. The role of mechanoregulatory factors in disease progression remains unclear. Through multiphoton second harmonic generation microscopy, we observed significant reorganization of the extracellular matrix (ECM) within psoriatic dermis. Collagen fibers were highly aligned and formed thick, elongated bundles, although overall fiber density was reduced, especially in the dermal papillae extending into the epidermis. Additionally, the ECM-modifying enzyme LOX was significantly upregulated in the dermis of psoriasis patients. In vitro experiments revealed a novel connection between HIF-1 stabilization and LOX protein regulation in mechanosensitive skin fibroblasts. LOX secretion and activity were directly linked to substrate stiffness, but were independent of hypoxia and IL-17. Furthermore, scRNA-seq analysis identified skin fibroblasts with high LOX expression and confirmed increased HIF-1 expression in psoriasis. These findings suggest a previously unrecognized mechanical aspect of psoriasis, with dysregulated mechanical forces potentially contributing to a positive feedback loop in fibroblasts, promoting tissue stiffening, reduced skin elasticity, and exacerbating disease pathogenesis.

Session 4,4

Neuropeptides in Borrelia burgdorferi transmission and skin infection

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Introduction: Erythema migrans (EM) represents the first and most common manifestation of Lyme disease and is caused by the tick-transmitted pathogen Borrelia burgdorferi. Untreated infections can cause local and systemic neurologic manifestations, including Acrodermatitis chronica atrophicans (ACA)-associated dysesthesia. However, it remains unclear if and how dermal nerve fibers are affected in different stages of infection.

Methods: This study investigates interaction of B. burgdorferi spirochetes with dermal nerve fibers within acute and chronic B. burgdorferi skin infection. Furthermore, it explores the expression of Calcitonin Gene-Related Peptide (CGRP), a neuropeptide known to influence neurogenic inflammation, its receptor, and the Nerve Growth Factor (NGF) as markers of neurogenic modulation in B. burgdorferi skin infection, comparing findings with skin affected by tick bites and healthy skin.

Results: We detected B. burgdorferi spirochetes to co-localize with cutaneous nerve structures (UCHL1+ Schwann cells) in infected skin. In parallel, the neuropeptide CGRP was increased in B. burgdorferi skin infection compared to healthy skin controls, and significantly upregulated around dermal nerve fibers co-localized with B. burgdorferi, indicating neuropeptide release caused by B. burgdorferi attachment. Single-cell RNA sequencing analysis reveals upregulation of the CGRP receptor CALCRL in the acute B. burgdorferi skin infection, particularly within antigen-presenting cells, T cells and natural killer cells, which are known to be repolarized upon binding of CGRP.

Conclusions: Overall, this ongoing study identifies upregulation of neuropeptides in the skin in response to B. burgdorferi infection suggesting neurogenic modulation.

Session 5,1

Ultraviolet radiation and air pollution induce melanocyte senescence and contribute to human skin aging

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Introduction: Extrinsic skin aging results from exposure to environmental factors such as sunlight, air pollution, and cigarette smoke. Melanocytes, which primarily protect the skin from ultraviolet (UV) light, become less active with aging. However, senescence of melanocytes and their contribution to skin aging has been insufficiently explored. Understanding the molecular mechanisms of melanocytes in response to environmental factors will help improve the development of novel therapeutics for aging and age-related pigmentation disorders.

Methods: We exposed human neonatal melanocytes and skin explants to UV (UVA+UVB), urban particulate matter (UPM), and a combination of both (UV+UPM), to understand how these environmental stressors affect skin biology and, in particular, melanocytes' homeostasis. Following melanocyte treatment, we investigated several morphological and physiological parameters such as cell proliferation, senescence state, apoptosis, pigmentation, DNA damage, and mitochondrial function. Treated skin explants were analyzed for signs of aging, including skin thinning, pigmentation alterations, and skin barrier function.

Results: Melanocytes demonstrated diverse responses to the different treatments in terms of senescence markers, cell survival, and pigmentation. UV exposure induced cellular senescence, whereas the combination of UV and UPM led to excessive DNA and mitochondrial damage that consequently resulted in cell death of melanocytes. Interestingly, UV and UV+UPM treatment caused a downregulation of the main melanogenesis-regulating transcription factor in the early stress phase, indicating a modulation of the melanogenesis process. Accordingly, UV+UPM-treated skin explants exhibited epidermal thinning, increased number of pyknotic nuclei, impaired skin barrier function, and altered pigmentation, all characteristic features of aged skin.

Conclusion: This new experimental setup will allow us to perform further research on mechanisms of extrinsic skin aging, including the role of melanocytes in this process. The findings potentially unveil novel insights into the role of melanocytes in the skin and the development of new therapeutical targets for pigmentation disorders and premature skin aging.

Session 5,2

Chronological maturation of the skin immune barrier is topographically different

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Introduction: Adult skin varies across regions, with differences in chemical, physical, microbiota, and immune barriers. However, data on immune differences in other age groups are limited. This study aimed to explore the chronological maturation of the immune barrier in various skin regions.

Methods: A TaqMan low-density array and immunohistochemical and immunofluorescence detection of various immune cells and mediators in sebaceous gland-rich (SGR) and gland-poor (GP) healthy skin were performed in children, adolescents, and adults.
Results: The maturation of SGR skin showed a significant increase in the mRNA levels of Th17-related molecules (IL-17A, IL-1B, RORC, LCN2) from childhood to adulthood, but with only a slight increase between childhood and adolescence. Additionally, T cell, Treg, dendritic cell (DC), and macrophage counts, plus the levels of several Th17-related protein (IL-17, IL-10, IL-23, CCL20, S100A8, sfTSLP) increased prominently with age. In GP skin, sfTSLP and AHR mRNA levels decreased, while T cell, and DC numbers, and Th17-related protein levels increased, although only moderately. Comparing the two barriers, SGR and GP skin were similar in childhood, with differences emerging in adolescence and becoming significant in adulthood, particularly in the IL-17 pathway, mainly produced by Th17 cells.

Conclusion: Our results show a similarly directed maturation process in GP and SGR regions, with more robust development in SGR skin immune barrier during and after the adolescence years. This is probably linked to the significant changes in the chemical and microbiota barriers of SGR skin during adolescence, and may explain the common occurrence of inflammatory skin diseases on teenage SGR skin, and underline the need for targeted skin care of this region.

Session 5,3

Tick feeding triggers lymph node homing of tolerogenic human epidermal Langerhans cells resulting in altered T cell activation

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Epidermal Langerhans cells (LCs) are the first antigen presenting cells potentially responding to tick vector feeding on human skin. LCs are crucial to shape the immune response by either inducing tolerance to harmless (self) peptides or an immune response to foreign antigens. However, the mechanism of LC polarization during tick feeding is still not well understood. To address this, we explore human LC polarization and subsequent T cell activation in response to tick saliva (TS) and Borrelia (B.) burgdorferi infection, using single-cell RNA sequencing, multi-color flow cytometry, immunofluorescence and organoid cultures.
We observed strong migration of epidermal LCs to the lymphatics after clinical tick bites and in an in vitro experimental tick bite model using TS injection. Emigrating LCs over-expressed the migration marker CXCR4 as well as the lymph node homing molecule CCR7, indicating their capability for lymph node homing. In line with this, monocyte-derived LCs (moLCs) stimulated with TS showed increased potential to invade CCL19 supplemented collagen gels. Additionally, when incubated with TS or B. burgdorferi, moLCs exhibited a tolerogenic phenotype characterized by the upregulation of the transcription factors IDO1 and IRF4. In lymph node organoids, tolerogenic LCs skewed polarization of autologous, naïve CD4+ T cells towards regulatory and type-2 helper T cells. This was verified by single-cell RNA sequencing of patients with Lyme borreliosis, where LCs exhibited increased expression of migratory molecules CXCR4 and CCR7, and tolerogenic transcription factors IRF4, IDO1 as well as IL4I1. Further, T cell composition was comparable to healthy skin from the same individuals.

Collectively, our results show that tick-feeding induces homing of tolerogenic LCs to the lymphatic system and subsequent skewed effector T cell polarization, resulting in an impaired immune response to tick-borne pathogens. These findings explain the low immunogenic response and high transmission rates of Lyme disease and other tick-borne infections.

Session 6,1

Statins to fight aggressive skin cancer?

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Background: Although cutaneous squamous cell carcinoma (SCC) rarely metastasises in the general population, tumours in patients with the hereditary condition recessive dystrophic epidermolysis bullosa (RDEB) manifest at an earlier age with devastating morbidity and are the cause of death in over 90% of patients. As clinicians have few treatment options with limited efficacy, these exceptionally aggressive tumours warrant the exploration of new approaches to identify therapeutics. In a transcriptome-guided drug repurposing effort, we found a surprising anticancer drug candidate class: statins – widely prescribed to treat high cholesterol with an excellent safety profile. To evaluate the potential of repurposing statins as a treatment for skin cancer, we performed mechanistic studies to elucidate both how statins can interfere with cellular tumour programmes, and why statins may have had equivocal results in anti-cancer studies to date.

Results: In our study, RDEB-SCC cells were treated with escalating doses of statins in viability and toxicity assays. A clear cytostatic effect of statins was demonstrated with IC50 values in the micromolar range. Through rescue experiments, it was revealed that statins inhibit the elevated proliferative potential of SCC cells by specifically targeting the mevalonate pathway. Our data suggest that mevalonate-derived isoprenoids are required by tumour cells to drive MAPK signalling via RAS prenylation. As a result, statins not only slow tumour growth, but also partly reverse the epithelial-to-mesenchymal phenotypic switch of tumour cells by interfering with their cellular metabolic programme. Using RNAseq transcriptome data from time-course as well as results from drug holiday experiments, we were able to identify a potential strategy tumours use to evade treatment via Sterol Regulatory Element-Binding Transcription Factor-1 activation.

Conclusion: Our findings provide encouraging evidence of how statins control cancer cell growth in RDEB-SCC with implications for the effective use of statins.

Session 6,2

Investigation of Metabolic and Secretory Pathways Driving Cutaneous Squamous Cell Carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is a prevalent non-melanoma skin cancer associated with high morbidity and mortality worldwide. Its development is a multistep process requiring genetic and epigenetic alterations, driven by risk factors such as UV radiation and chronic inflammation. Although much focus has been placed on the epidermal keratinocytes in cSCC pathogenesis, the role of dermal fibroblasts and inter-tissue communication remains underexplored. UV radiation impacts both epidermal and dermal cells, activating pathways related to growth, differentiation, senescence, and inflammation. Importantly, the accumulation of senescent cells, with their altered secretome known as the senescence-associated secretory phenotype (SASP), plays a critical role in skin aging and cancer progression.

We have shown that NIX-dependent mitophagy in dermal fibroblasts is essential for mitigating UVB-induced damage and maintaining homeostasis. Knockdown of NIX impairs mitophagy, induces cellular senescence, and triggers the release of extracellular vesicles (EVs) containing mitochondria. Our studies using 3D skin equivalents incorporating NIX-depleted fibroblasts revealed keratinocyte hyperproliferation and differentiation defects, mimicking early cSCC development. In contrast, depletion of GDF15, a cytokine upregulated in senescent cells, resulted in epidermal thinning, reinforcing the notion that fibroblast-secreted factors modulate epidermal homeostasis.

These findings suggest that distinct SASP components, secreted by senescent fibroblasts, dictate epidermal phenotypes, with GDF15 depletion leading to skin aging and NIX depletion promoting keratinocyte dedifferentiation and aberrant proliferation. This study aims to further explore how NIX-dependent mitophagy and the fibroblast secretome contribute to the pathogenesis of cSCC and how trans-tissue communication influences epidermal changes associated with aging and tumorigenesis.

Session 6,3

Adoptive cell therapy using skin resident gamma delta T cells to treat cutaneous squamous cell carcinoma.

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Gamma delta (γδ) T cells play important roles in the surveillance of cellular stress, tumors, and infection, that help maintain tissue integrity and modulate adaptive responses to these stimuli. γδ T cells can recognize malignant cells via surface molecules and display killing activity upon activation. γδ T cells respond to a variety of solid and hematological tumors in vitro and in in vivo xenograft models, and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. In clinical trials, adoptive transfer of ex vivo expanded γδ T cells lead to temporary tumor regression and increased survival of leukemia patients. Hence, γδ T cells are promising candidates for anti-tumor immune therapeutic approaches. Due to technical difficulties to isolate enough γδ T cells from human skin, most studies on cutaneous γδ T cell biology were focused on murine skin resident γδ T cells. Here we are using novel methodologies to expand functional cutaneous γδ T cells ex vivo. Upon adoptive transfer of these cells into mice that have received skin tumors, we can study their migration, maintenance, and phenotypic adaptation in vivo. Specifically, we have established a cutaneous squamous cell carcinoma (SCC) xenograft mouse model in which the grafted SCC tissue resembles tumors of patients macroscopically and microscopically. In this model, γδ T cells engrafted and are maintained in the tumor tissue. Crucially, these cells displayed an activated and cytotoxic phenotype after isolation from the tumor mass. This model enables in depth and mechanistic studies of the biology of cutaneous γδ T cells in the tumor microenvironment. Additionally, the in vivo tumor model can be utilized to study the therapeutic potential of γδ T cells in cutaneous carcinomas, paving the way to novel anti-tumor treatments.

POSTERS

P01

Patient with ADAM17 mutation-caused Neonatal Inflammatory Skin and Bowel Disease 1 responds to personalized treatment with IL-12B-inactivating antibody therapy (Ustekinumab)

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Background: Neonatal inflammatory skin and bowel disease 1 (NISBD1) is a rare disease caused by homozygous or compound heterozygous mutations in the ADAM17 gene, which encodes for a disintegrin and metalloproteinase 17. To date, only five families with this disease have been reported.

Objectives: We studied a new case of NISBD1 with a homozygous point mutation in ADAM17 to detail the clinical, histopathologic, and immunological phenotype as well as to identify possible treatment options in this rare disease. Furthermore, we studied the effect of the new ADAM17 point mutation in situ and in vitro in detail.

Methods: We employed whole exome sequencing, histopathologic and immunofluorescent studies as well as gene and protein expression analysis. We performed in silico and in vitro mechanistical analyses of the ADAM17 point mutation. Furthermore, immunologic studies using FACS analysis of lesional skin, gut and blood of the patient were conducted.

Results: The 23-year old patient harboring a new ADAM17 point mutation presented with erythroderma and scaling since birth. Using in silico predictions and in vitro activity assays, we found that the patient's point mutation severely affects the function of ADAM17. Performing cytokine profiling of the patient's lesional skin, we found a significant upregulation of IL-12B. Systemic therapy with an antibody targeting IL-12B (ustekinumab) was initiated leading to an amelioration of skin manifestations.

Conclusion: With this case report, we create evidence for the effect of ADAM17 impairment on the immune system, and provide a sound example of personalized precision medicine. Further studies are needed to evaluate the long-term and potentially universal benefit of this treatment modality in NISBD1 and to elucidate the exact mechanism of ADAM17 impairment on the immune system.

P02

The proteome of keratinocyte cultures from different types of non-lesional psoriatic skin exhibits distinct characteristics.

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Background: The hyperproliferation of keratinocytes plays a key role in the appearance of plaques in psoriasis. Our previous studies have shown that psoriasis severity-related alterations can be observed in non-lesional skin areas of untreated patients, and abnormalities have also been observed in keratinocytes of previously-lesional clinically resolved skin of treated patients. We hypothesized that keratinocytes from different psoriatic non-lesional (PS-NL) skin types may have different cell biological properties and therefore aimed to explore these differences.

Methods: We performed a whole proteomic analysis of keratinocyte cultures from healthy, untreated mild- and severe-PS-NL, and resolved (RES-PS-NL) skin areas. Reactome over-representation tests were performed with differentially expressed proteins (DEPs, with twofold changes). Morphological differences of cells were investigated by field emission scanning electron microscopy (SEM). Behavioral differences were investigated by MTT assay, and in wound healing model by CytoSmart time-lapse microscopy.

Results: Based on the whole protein expression pattern and the number of DEPs, the highest difference was observed between healthy and resolved PS-NL keratinocytes, and the highest similarity between mild-PS-NL and RES-PS-NL cells. DEPs with increased expression in the all three PS-NL groups, mainly affected oncogene-induced senescence, while decreasing DEPs in the same group mostly influenced the mitotic cell cycle process. We observed that, in RES-PS-TM versus healthy keratinocytes had slower wound closure and the expression of wound healing proteins also showed mainly different pattern. MTT assay values also showed a decreasing trend in RES-PS-TM versus healthy cells. SEM analysis revealed that the presence of filopodia and lamellopodia was mainly characteristic for mild-PS-TM and RES-PS-TM keratinocytes.

Conclusions: Our results suggest that PS-NL keratinocytes may be able to contribute to the healthy-looking appearance of the PS-NL skin with different mechanisms, in which RES-PS-TM showed the greatest difference among the PS-TM keratinocytes with alterations related to wound healing.

P03

Clinical Evaluation of Alpha-1-Acid Glycoprotein as a Novel Biomarker for Malignant Melanoma

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Introduction & Objectives: Melanoma, characterized as the deadliest skin cancer with a high propensity for metastasis and a rising incidence rate, underscores the critical importance of early detection for improved long-term outcomes. Consequently, there exists a pressing need for diagnostic biomarkers conducive to early tumor identification. This study endeavors to investigate the diagnostic utility of Alpha-1-Acid Glycoprotein (AGP), an abundantly glycosylated serum acute-phase protein, which may be shed by tumor cells, as a prospective biomarker for malignant melanoma.

Materials & Methods: Serum AGP from patients afflicted with high-risk primary melanomas and healthy controls underwent meticulous isolation and purification to eliminate confounding factors. Subsequently, oligosaccharide side chains were liberated and chemically modified. The resultant derivatives were analyzed using a hydrophilic interaction liquid chromatography–tandem mass spectrometric approach. Linear discriminant analysis (LDA) was employed to scrutinize the acquired data. Additionally, serum S100B concentrations were determined for comparative purposes, enabling the assessment of sensitivity, specificity, as well as negative and positive predictive values.

Results: Within the melanoma cohort, a conspicuous upregulation of fucosylated glycans was observed, particularly those exhibiting greater branching, with this upregulation appearing to correlate positively with the number of attached fucose units. From a multitude of parameters, those displaying the highest discriminatory potential were meticulously selected. Discriminatory power rankings of the isomers were established to distinguish between the groups. LDA elucidated clear demarcation between control and melanoma samples. Upon cross-validation, these findings were compared with S100B protein concentrations in the studied population, revealing significantly enhanced sensitivity compared to S100B.

Conclusion: This investigation underscores the diagnostic relevance of alterations in the glycan profile of human serum AGP in melanoma detection. In conclusion, a novel potential biomarker has been identified, exhibiting markedly superior performance to the established serum marker S100B, particularly in terms of sensitivity and negative predictive capability within the studied population.

P04

Unique Trm Cells Define Atopic Dermatitis and Psoriasis

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Aim: Atopic dermatitis (AD) and Psoriasis (PSO) are chronic inflammatory skin diseases characterized by significant T-cell infiltration. Our goal was to find the cytokine producing T-cell types and analyze their differences between these conditions.

Methods: Previously published CITE-seq analysis of CD45+ cells revealed distinct Trm cell characteristics in atopic dermatitis (AD) and psoriasis (PSO). We reclustered T cell subsets and characterized Trm cells in each disorder using Seurat and Harmony R packages for batch effect correction. Tmem cell subsets (Trm, Tcm, Tmm, Tem) were defined based on CD3, CD4, CD8, CD69, CD103, CD49a, CD197, and CD62L expression.

Results: CD4 and CD8 Trms were significantly increased in AD and PSO compared to controls. CD8 Trms showed similar ratios, while PSO had a higher CD4 Trm ratio. Trms were the primary source of cytokines, with AD-specific CD4+ Trms producing Th2 cytokines (IL-13), and PSO-specific CD8+CD103+ Trms producing Th17 (IL17A, IL17F) and Th1 (IFNG) cytokines. Both CD4+ and CD8+ Trms in AD showed higher mobility, potentially recirculating from skin periphery. Trm cells also differ in their co-signaling molecules, with AD Trm clusters showing elevated expression of co-stimulatory molecules, while PSO Trm clusters exhibits increased expression of mainly co-inhibitory molecules.

Conclusions: These findings may provide insight into the clinical picture of these two inflammatory skin diseases. Atopic dermatitis (AD) lesions are characterized by blurred edges, possibly due to Trm cells leaving the skin and returning to form lesions in different areas. In contrast, Trm cells in psoriasis show lower expression of migration and egress genes, remaining more localized. As a result, psoriasis lesions tend to have well-defined edges and frequently recur in the same location.

P05

Impact of ultraviolet B on the circadian system

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Many UV-induced processes are under circadian control, however, the relation and interaction of UV radiation and circadian rhythm are less well understood in human skin. The circadian system following UVB exposure was investigated to detect diurnal variations in the effects of radiation. Moreover, the diurnal protective effect of sunscreens was also examined. Volunteers were recruited to investigate the effects of UVB radiation according to the following treatment plan: UV treatment was applied in the morning (7h) and afternoon (15h), followed by MED determination 24 h later. Subsequently, a fourfold MED (4MED) was applied (7 and 15h) to untreated and sunscreen-treated skin areas. Skin biopsies were obtained at both time points from control areas and 24 hours after exposure from 1MED, 4MED, and sunscreen-treated 4MED irradiated areas. Global gene expression was measured by RNA sequencing, followed by differential expression and functional enrichment analyses to identify UV-modulated genes. Similar gene expression patterns were obtained in the untreated control and in skin treated with sunscreen and four times the UVB dose. While similar pathways are regulated in both UVB treatments, the dosage effect is shown by the higher number of dysregulated genes under 4MED treatment. Key pathways include TGF beta signaling, defense response, and several structural components (FLG, IVL, SPRR genes). Investigating genes that are differentially regulated between control/treatments according to the time of day, we find that while circadian rhythm genes are fairly consistently changing in all conditions, there are specific treatment-related alterations. Examples include genes where the normal morning-afternoon circadian upregulation is dampened (PER3, NR1D2); normal upregulation is canceled by treatment (RBM14); or in some cases where the circadian trend is reversed (KRT31). While the observed fold changes are modest, the change or reversal in regulation suggests widespread effects of treatment on the normal circadian rhythm.

P06

Cutibacterium acnes alters keratinocyte immune behavior by activating innate immune memory events

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External insults can transiently activate various cutaneous cell types, and innate immune and inflammatory activation may not disappear without a trace. This referenced to as trained immunity or innate immune memory (IIM). The cutaneous microbiota is in constant contact with the keratinocytes, which sense them through pattern recognition receptors. We were interested in determining whether Cutibacterium acnes (C. acnes) is capable of inducing a persistent IIM in these cells. We used C. acnes for primary training, and after five days of resting, Pam3CSK4 (TLR1/2 agonist) for secondary induction in normal human epidermal keratinocytes of breast (NHEK-B) and abdominal (NHEK-A) origin. We found significantly higher expression levels of selected immune effector genes (e.g., TNFα and IL-8) in NHEK-B cells, but lower in NHEK-A samples, indicating region-specific innate training vs. tolerance. The global 5-methylcytosine (5mC) and 5 hydroxymethylcytosine (5hmC) content of genomic DNA was reduced in trained, uninduced NHEK-B cells compared to NHEK-A cells, supported by the observed region-specific mRNA expression differences of TET and DNMT genes. C. acnes training affected the metabolism of NHEK cells. We found decreased lactate production in the untrained vs. trained NHEK-A and-B cells, in the absence of secondary induction. Pam3CSK4 treatment caused an increase only in the C. acnes-trained NHEK-B ones. Our microbiota induces region-specific IIM-like changes in the skin, and the underlying signaling, epigenetic, and metabolic changes may account for the differences between regionally distinct NHEK cultures.

P07

Identification of clones by T-cell receptor Vβ repertoire analysis supports diagnosis of leukemic cutaneous T-cell lymphoma

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The group of erythrodermic leukemic cutaneous T-cell lymphoma (L-CTCL) comprises Sézary syndrome (SS) and progressive erythrodermic mycosis fungoides (MF) with infiltration of blood. Differentiation of L-CTCL from erythrodermic conditions of other origin sometimes bears difficulties. Identification of T-cell clones by TCR (T-cell receptor) Vβ repertoire analysis might support diagnosis of L-CTCL.

Between March 2017 and June 2024, whole peripheral blood mononuclear cells (PBMCs) were isolated from the blood of 65 patients (23% female; age: 66±17 years). In 19 cases, patients were diagnosed with L-CTCL; 19 patients had erythroderma of a different origin; 27 patients presented with non-erythrodermic skin diseases. Patients' PBMCs were analysed for T-cell clones using a multiparametric analysis tool designed for quantitative determination of the TCR Vβ repertoire of T-lymphocytes via flow cytometry (Beckman Coulter #PN IM3497, USA).

Frequencies of the dominant TCR Vβ types within the detectable and undetectable Vβ repertoire were significantly higher in L-CTCL patients than in patients with non-leukemic skin conditions ($p<0.001$, Wilcoxon signed rank test). As identified via Youden index, 24% represented the optimal cut-off value to differentiate between the groups (sensitivity: 94.7%, specificity: 95.7%).
The calculation of receiver operating characteristic (ROC) curves indicated higher diagnostic accuracy for TCR Vβ repertoire analysis (AUC=98.6%) compared to flow cytometry using conventional panels (AUC=88.7%) without reaching statistical significance, however ($p=0.066$, DeLong's test for two ROC curves).

TCR Vβ repertoire analysis represents a highly sensitive and specific method to detect malignant clones in the blood of patients with L-CTCL. The methodology also allows screening for targetable molecules such as CCR4, CD30, CTLA-4 and CD52.

P08

Identifying Aggressive Histological Subtypes of Basal Cell Carcinoma Utilizing Dermoscopically Guided High-Frequency Ultrasound

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Introduction: Basal cell carcinoma (BCC) is the most common type of skin cancer. The histological subtype (HST) of BCC, essential for determining treatment strategies currently relies on invasive biopsy. This study aims to assess the potential of dermoscopically guided high-frequency ultrasound (DG-HFUS) imaging in the differentiation of aggressive HST BCCs.

Methods: This prospective study involved clinical and dermoscopic evaluations of BCCs, followed by DG-HFUS imaging at 33 MHz, surgical excision, and histopathological analysis. 75 patients with 78 BCCs were enrolled with 63 lesions used to develop a novel DG-HFUS risk classification algorithm and 15 lesions for algorithm validation. The mean age of the cohort was 72.9 ± 11.2 years, with histological analysis identifying 16 lesions as aggressive HST (infiltrative or micronodular subtypes) and 47 as low-risk HST (superficial or nodular subtypes). Statistical analysis included a one-sided Fisher's exact test for categorical assessment and Receiver Operating Characteristic (ROC) curve analysis for evaluating diagnostic accuracy.

Results: DG-HFUS effectively distinguished aggressive BCC HSTs by detecting irregular shapes ($p < 0.0001$), ill-defined margins ($p < 0.0001$), and non-homogeneous internal echoes ($p = 0.004$). A risk-categorizing algorithm was developed, demonstrating superior sensitivity (82.4%) and specificity (91.3%) compared to conventional macroscopic and dermoscopic evaluations (sensitivity: 40.1%, specificity: 73.1%). Dermoscopic assessment yielded positive and negative predictive values (PPV and NPV, respectively) of 30.2% and 76.8%, while the DG-HFUS-based algorithm showed a PPV of 94.7% and an NPV of 78.6%. Validation of the algorithm with an independent image set (n = 15), evaluated by two blinded assessors, revealed a sensitivity of 83.33% and specificity of 91.66%.

Conclusion: We demonstrated that our novel algorithm based on core DG-HFUS features can distinguish high-risk BCC HSTs with higher sensitivity and specificity compared to combined dermoscopic and macroscopic assessment. This improved precision in early risk categorization harbors clinical implications for BCC management.

P09

Investigation the efficacy of doxycyclin prophylaxis in preventing bacterial STIs

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Introduction: Sexually transmitted infections (STIs) remain a global health issue, with increasing incidence rates. This trend has been particularly pronounced among men who have sex with men (MSM) and transgender women (TGW). Our goal was to investigate the effects of doxycycline pre- and post-exposure prophylaxis (doxy-PrEP/PEP) on bacterial STIs by conducting a systematic review and meta-analysis.

Methods: We systematically searched PubMed, Embase, and CENTRAL for randomized controlled trials. Our primary endpoint was the incidence of bacterial STIs. A random-effects model was used to estimate pooled effect sizes.

Results: We identified six eligible studies, comprising data from seven articles and four conference abstracts, focusing on MSM, TGW, and cisgender women. A pooled analysis of 1,766 participants, including 602 newly diagnosed STIs, demonstrated a 56% reduction in overall STI incidence with the use of doxy-PrEP/PEP (RR = 0.44; 95% CI: 0.30-0.65; I^2 = 73%). With doxy-PEP, including MSM and TGW only, the overall STI incidence decreased by 60% (RR = 0.40; 95% CI: 0.28-0.57; I^2 = 37%). In this subgroup, the incidence of chlamydia decreased by 81% (RR = 0.19; 95% CI: 0.08-0.44; I^2 = 39%), syphilis by 77% (RR = 0.23; 95% CI: 0.14-0.36; I^2 = 0%), and gonorrhoea by 45% (RR = 0.55; 95% CI: 0.34-0.87; I^2 = 41%). One serious adverse event was reported.

Conclusion: Doxy-PEP significantly reduces the incidence of chlamydia and syphilis and demonstrates potential efficacy against gonorrhoea, contingent on local resistance patterns. It represents a promising strategy for bacterial STI prevention in MSM and TGW populations.

P10

Novel variants in medium and low penetrance melanoma predisposing genes in a Hungarian familial melanoma malignum cohort

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Introduction

According to our current knowledge, the genetic predisposition of melanoma is defined by germline and somatic variants. Germline variants involve the presence of rare pathogenic or likely pathogenic variants of high, medium and low penetrance melanoma predisposing genes. Rare variants of high penetrance melanoma predisposing genes are associated with primary disease-causing role in melanoma development, while rare ones of medium and low penetrance predisposing genes can significantly increase the personal melanoma risk.

Methods: Our aim was to clarify the germline genetic background of a Hungarian familial melanoma cohort (n=17) using a gene panel of 30 melanoma predisposing genes.

Results: Germline genetic variants were identified in 10 (58.82%) patients of the 17-member Hungarian cohort. A novel, likely pathogenic, missense variant, the p.Y143C in a medium penetrance melanoma predisposing gene, melanocortin 1 receptor gene (MC1R) and two novel, likely pathogenic, nonsense variants in low penetrance genes, the p.Q218Ter in caspase 8 gene (CASP8) and the p.Q40Ter in fat mass- and obesity-associated gene (FTO) were detected. Family screenings of the identified variants are currently ongoing.

Conclusions: This study highlights the importantance of the elucidation of the germline genetic background of melanoma, which may help us to better estimate individual risk and the risk of family members and to optimize preventive, screening and therapeutic measures for each melanoma patient.

P11

Host Biomarkers and Antibiotic Tissue Penetration in Sepsis

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Introduction: Sepsis-induced pathophysiological changes may lead to pharmacokinetic variability that alters antibiotic concentrations at the host infection site. This poses a challenge in clinical practice, as sufficient antibiotic concentrations in tissue are necessary to eradicate bacterial pathogens effectively. In this exploratory study, we aimed to evaluate the potential of routinely used laboratory biomarkers to predict subcutaneous and muscle tissue penetration of moxifloxacin in septic patients.

Methods: We retrospectively analyzed data from 10 septic patients included in a pharmacokinetic study, in which moxifloxacin concentrations in subcutaneous adipose and muscle tissues were measured with microdialysis. We correlated the tissue-to-plasma ratio with various clinical biomarkers.

Results: Our results revealed significant correlations for CRP, LDH, BUN, GPT and total protein with moxifloxacin subcutaneous penetration and BUN, GOT and GPT with muscle penetration. Notably, all biomarkers except CRP correlated negatively with tissue penetration. Moreover, we found a positive correlation between moxifloxacin protein binding and total plasma proteins and albumin.

Conclusion: Biomarker tissue correlations suggest that the penetration of moxifloxacin into tissues is a complex process influenced by factors like inflammation, tissue integrity, liver function, protein levels, and renal function. Understanding these interactions might help optimize antibiotic dosing strategies.

P12

Unlocking the potential of tumour necrosis factor inhibitors on the risk of atherosclerotic cardiovascular events in immune-mediated inflammatory diseases

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Introduction: Increased risk of cardiovascular (CV) events is associated with immune-mediated inflammatory diseases (IMIDs), like psoriasis (Pso), inflammatory arthritides, or inflammatory bowel diseases. The excess CV risk may be facilitated by tumour necrosis factor (TNF)-α-mediated immunological pathways involved in the pathomechanisms of both IMIDs and atherosclerosis. Through the inhibition of the shared inflammatory pathways, TNF-α inhibitors (TNFis) can have a superior risk-reducing effect on CV events compared to the conventional systemic non-biological (CSNB) treatments, which do not directly act on TNF-mediated pathways. Our objective was to systematically investigate the effects of TNFis compared to CSNBs in IMIDs on the risk of atherosclerosis-derived CV events.

Methods: A systematic search was conducted in three databases. Randomised controlled trials, cohort, and case-control studies were eligible with the outcome of the incidence of atherosclerotic CV events in TNFi-treated patients compared to patients receiving CSNBs in IMIDs. Random-effect meta-analysis of pooled fully adjusted multivariate hazard ratios (HRs) and incidence rate ratios (IRRs) was performed with major adverse cardiovascular events (MACE), myocardial infarction (MI) and cerebrovascular events (CeVE) as primary main outcomes.

Results: The systematic search resulted in 8724 hits. 56 articles were included in total, of which 29 studies were eligible for the quantitative analyses. The TNFi-treated group had a significantly lower risk of MACE compared to the CSNB-treated group (HR= 0.74, 95% confidence interval (CI) 0.58–0.95; IRR= 0.77, 95% CI 0.67–0.88). The clinically and statistically significant benefit of TNFis on MACE reduction was also demonstrated in the subgroup of PsO and psoriatic arthritis patients. The superiority of TNFis compared to the CSNB group was also shown for the outcomes of MI and CeVE in the analyses of IMID patients.
Conclusion: Prior use of TNFis instead of CSNBs in the therapeutic line can be recommended to decrease the risk of atherosclerotic CV burden in IMIDs.

P13

Dysregulated T cells in skin cancer associated with recessive dystrophic Epidermolysis bullosa

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The skin of patients with Epidermolysis bullosa (EB) is highly fragile due to inherited mutations in genes that encode structural proteins with roles in keratinocyte integrity and cellular adhesion. Minimal trauma causes mucocutaneous blister formation in these patients and skin damage often leads to the formation of chronic wounds. Highly aggressive cutaneous squamous cell carcinoma (SCC) that typically arise in these chronic wounds are the main cause of death in patients with recessive dystrophic EB (RDEB). We discovered that skin-tropic as well as tumor-infiltrating cells were dysfunctional in RDEB patients that were diagnosed with an SCC. Particularly, IL-17-producing CD4+ T cells were elevated within the tumor tissue and in consequence we found IL-17A-induced genes significantly enriched within RDEB SCC tumor cells. In line with this, we showed that IL-17 promoted the growth of SCC cells in a xenograft model, suggesting that interfering with IL-17 signaling might be a therapeutic option in RDEB patients with SCC.

Additionally, we revealed that γδ T cells, a key T cell type with anti-tumor activity, showed signs of dysfunction in RDEB patients with SCC. Using a preclinical SCC model, we found that adoptively transferred human cutaneous γδ T cells could control SCC tumor growth, underscoring their therapeutic potential to treat skin cancer.

P14

scRNA-seq Pathway Analysis with ReactomeGSA

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Background: ReactomeGSA is a widely used platform for multi-omics pathway analysis within the Reactome knowledgebase, allowing comparisons across various 'omics technologies. However, quantitative pathway analysis for single-cell RNA sequencing (scRNA-seq) data has not been supported due to algorithmic challenges. Here, we introduce a novel feature that enables large-scale scRNA-seq pathway analysis within ReactomeGSA, overcoming previous performance and algorithmic limitations.

Methods: To support quantitative pathway algorithms for scRNA-seq data, we implemented methods to convert scRNA-seq data into pseudo-bulk datasets. Enhancements to the ReactomeGSA R package enable aggregation and sub-clustering of cells within clusters, using techniques such as random aggregation, variable-based aggregation, and sub-clustering. These pseudo-bulk datasets allow quantitative pathway analysis within the Reactome framework. We benchmarked the approach by applying it to public scRNA-seq datasets, assessing its performance in generating valid pathway results.

Results: Our data shows that generating pseudo-bulk data enables quantitative pathway analysis for scRNA-seq data within ReactomeGSA. All tested aggregation and subclustering methods produced biologically meaningful pathways. Aggregation methods outperformed sub-clustering in terms of runtime and resource efficiency, with no significant advantage observed in the results from sub-clustering algorithms. Therefore, indicating that the size of the pseudo-bulk samples, rather than the algorithm used, affects pathway results and their significance.
Furthermore, integrating quantitative pathway analysis within Reactome allows us to offer advantages in terms of pathway visualization and performance scalability compared to existing methods.

Conclusions: The updated ReactomeGSA R package overcomes previous algorithmic challenges, enabling quantitative pathway analysis for scRNA-seq data for the first time. This update combines both the algorithmic advantages of quantitative pathway analyses, as well as the powerful visualizations offered by Reactome that are for the first time made available for scRNA-seq data.

P15

Exploring tBHP-induced senescence in melanocytes and its role in skin aging

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Background /Aims: Extrinsic skin aging results from exposition to environmental factors. The accumulation of senescent skin cells is a hallmark of aging and contributes to age-related diseases. Melanocytes, one of the main cell types found in the epidermis, contribute to the protection of the skin against environmental impacts mainly via pigmentation. This study aims to develop a model of cellular senescence in human melanocytes using tert-butyl hydroperoxide (tBHP) as an oxidative stress inducer. We sought to characterize the senescence phenotype of melanocytes by investigating how tBHP-induced senescence impacts key biological processes and how senescence of melanocytes induced by oxidative stress contributes to skin aging and pigmentation disorders.

Methods: Human melanocytes were treated with tBHP to induce senescence. The senescence phenotype was assessed by evaluating common senescence markers such as DNA damage, mitochondrial function, changes in expression of cell cycle regulators. Induction of cell cycle arrest was observed by monitoring growth inhibition and morphological changes. Additionally, we monitored melanogenesis, and examined the senescence associated secretory phenotype (SASP) to understand how senescence affects these processes.

Results: Our results demonstrated that tBHP treatment induces a shift in melanocyte behavior towards a senescent phenotype, characterized by a marked inhibition of cell proliferation. Furthermore, senescent melanocytes showed alterations in morphology and cell size. Additionally, experiments targeting senescence markers demonstrated an increase in DNA damage identified with γH2AX and SAHF along with mitochondrial fragmentation. Various experiments showed increased levels of melanin in senescent melanocytes in response to stress induction.

Conclusion: tBHP-induced senescence in melanocytes is a valuable model for studying the underlying molecular mechanisms of skin aging. Future findings using this senescence model could pave the way for identifying potential therapeutic targets for skin aging and related pigmentation disorders. Further investigation is needed to better understand the full impact of senescence in melanocytes on skin aging.

P16

Reactive lipids from the secretome of senescent fibroblasts modify the extracellular matrix with functional consequences for resident cells

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Collagen is the most abundant protein of the extracellular matrix in the skin and its degradation and modification lead to visible signs of skin aging. We found specific chemically reactive lipids accumulated in senescent dermal fibroblasts, which have the potential to bind covalently proteins via Schiff Base or Michael Adduction. Our study aims to investigate the modification of collagen by lipids that were described to be part of the senescence-associated secretory phenotype (SASP).

Using mass spectrometry, we have identified collagen modification of 4-Hydroxynonenal (HNE) and other aldehyde compounds derived from the oxidation of polyunsaturated phospholipids (OxPAPC). We found that collagen modified by these SASP lipids changes the fate and functionality of skin resident cells. Macrophages display a modification-specific expression of cyto-/chemokines and functional impairment of Toll-like receptor signaling. Both HNE and OxPAPC modified matrices induce an early senescent phenotype in macrophages. Fibroblasts show an inflammatory signature, especially HNE modified matrix induced expression of IL6, IL8 and CXCL1. Keratinocytes cultured on modified basal lamina collagen IV display transient expression of HO1 and elevated IL8 levels. Additionally, lipid modified collagen used to generate organotypic skin equivalents led to disturbed differentiation of epidermal keratinocytes. Further, cells within these skin equivalents with modified dermal compartment show lower expression of LaminB1 while yH2AX is upregulated, which indicates a senescent phenotype. These findings shed light on how reactive lipids derived from senescent cells can modulate inflammation and senescence propagation.

P17

Establishment of an in vivo RDEB-SCC humanized xenograft mouse model to study the effect of IL-17A on tumor progression

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Epidermolysis bullosa (EB) is a group of rare genetic skin disorders with mutations in genes responsible for mucocutaneous structural integrity. Even minor mechanical insults result in blistering of skin and mucous membranes. Most patients with recessive dystrophic EB (RDEB), a severe form of EB, develop Squamous Cell Carcinomas (SCC), which are highly aggressive in nature. SCC in EB patients typically arise in inflamed chronic skin wounds. We thus hypothesize that cutaneous inflammation is promoting SCC tumors. In line with this notion, we found that the levels of IL-17A were significantly increased in RDEB patients compared to healthy controls both systemically (in plasma) and locally (in skin blister fluids). Additionally, the fraction of IL-17A producing skin tropic CD103+CLA+CD4+ circulating tissue-resident memory T cells (cTRM) was significantly increased in RDEB patients with SCC compared to EB patients without SCC or healthy controls. Finally, we observed elevated expression of IL-17A mRNA by T cells within RDEB SCC tissues compared to RDEB patient skin (in scRNA sequencing). We found that IL-17A promoted the growth of human RDEB-SCC xenografts in a mouse model where we injected primary RDEB-patient derived SCC cells (along with patient-matched cancer associated fibroblasts) into the skin of immunodeficient NSG mice.We further plan to utilize this model to study the role of IL-17A on the formation of metastases and to test the efficacy of IL-17A neutralization as a novel approach to treat the highly aggressive RDEB-SCC in patients.

P18

ServEB: Integrating clinical, morphological and molecular information using data science and AI to optimize clinical research and care in Epidermolysis bullosa

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Introduction: The servEB project combines clinical expertise, advanced statistical analyses, and AI-driven imagine recognition technology to advance Epidermolysis Bullosa (EB) research. By fostering collaboration between clinicians, natural scientists, statisticians, IT experts and patient representatives, the project aims to improve clinical trial outcomes, facilitate patient self-reporting through mobile devices, and apply multi-aspect statistical analysis to enhance data validity while reducing patient burden.

Methods: First, servEB focuses on applying AI tools to enhance existing whole-body 3D stereophotographic imaging. Imaging recognition tools will be assessed for their validity and level of automation to accurately detect and quantify EB lesions, such as blister size and lesional skin areas. Imaging data will be acquired via standardized clinical as well as patient-self photography. In parallel, a mobile app will be developed for the upload of clinical images and the collection of patient-reported data based on, e.g., pain and pruritus scores. Thereby remote monitoring can be facilitated, potentially enabling less frequent on-site visits and continuous monitoring while reducing patient burden. Finally, all collected data will be harmonized and analyzed using suitable statistical methods. These analyses will combine lesion metrics with patient self-assessments to gain comprehensive insights into treatment efficacy.

Results: AI-based algorithms for detection and quantification of affected skin areas are under development, with early 3D imaging results currently being evaluated. Comparative assessment of various statistical methods is intended to identify optimal models for analyzing relevant clinical outcomes. The patient mobile application is currently conceptualized by conducting an initial survey amongst patients, their representatives and caregivers, to reflect on the scope of the app and to gauge acceptance of digital data acquisition.

Conclusion: This project demonstrates the value of integrating AI tools and advanced statistical methods to enhance EB research, reduce patient burden, and improve data accuracy. These innovations will offer significant improvements in clinical trial design and patient care.

P19

Immunomodulatory effects of Selumetinib on RDEB-SCC cells

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Background: Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic skin disease caused by mutations in COL7A1, resulting in defective type-VII collagen. Patients are at high risk to develop aggressive squamous cell carcinomas (SCC), which are the leading cause of early death. Current treatment options for RDEB-SCCs are limited. Using a transcriptome-guided computational drug repurposing approach, we identified the MEK inhibitor Selumetinib to have anti-tumor efficacy in a xenograft mouse model. This study aimed to investigate the immunomodulatory potential of Selumetinib in RDEB-SCC cells.

Methods: The effects of Selumetinib on programmed death-ligand 1 (PD-L1) and major histocompatibility complex class I (MHC-I) protein expression were analysed using Western Blot. Cytokine expression levels were assessed by semi-quantitative real-time PCR (sqRT-PCR).

Results: Selumetinib treatment significantly downregulated PD-L1 expression, a critical factor in tumor immune evasion, and upregulated MHC-I expression, important for antigen presentation and T-cell-mediated immune responses. Furthermore, RDEB-SCC cells treated with Selumetinib showed decreased expression levels of pro-inflammatory cytokines, including interleukin-1β (IL-1β), transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α).

Conclusions: Selumetinib demonstrated immunomodulatory activity in RDEB-SCC cells by reducing PD-L1 expression and increasing MHC-I expression, thereby potentially improving tumor immunogenicity.

P20

The impact of ER stress on γδ T cells in the tumor microenvironment

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Gamma delta T cells (γδ T cells) are unconventional T lymphocytes with a role in the immune surveillance of cellular stress. γδ T cells respond to a wide range of tumors and tumor infiltrating γδ are the most significant favorable prognostic immune population among 39 cancer types. However, a suppressive tumor microenvironment (TME) can impair γδ function, and the mechanisms are not fully understood. Thus, we aim to elucidate cellular communication between tumor cells and tissue resident γδ T cells to provide a basis for the development of effective anti-tumor therapies. The TME is often characterized by increased endoplasmic reticulum (ER) stress, a cellular response to an accumulation of un-/misfolded proteins in the ER lumen that can be promoted by several environmental insults (e.g. hypoxia, glucose deprivation). To restore cell homeostasis, the ER activates pathways collectively known as unfolded protein response (UPR), and an enhanced UPR correlates with poor clinical outcomes in cancer patients. Crucially, the UPR can support tumor growth by regulating immune cell function. We hypothesize that ER stress is a mechanism used by tumor cells to dysregulate the activation and function of tumor-infiltrating cutaneous γδ T cells. To this end we induced ER stress pharmacologically in primary human cutaneous squamous cell carcinoma cell lines using thapsigargin and treated cutaneous γδ T cells with the conditioned medium of these cells. This induced the upregulation of activation and cytotoxic markers and concomitantly reduced the expression of NK cell receptors (NKRs) and the production of pro-inflammatory cytokines by γδ T cells. These findings support the hypothesis that ER stress alters γδ T cell responses in the TME, offering new insights for optimizing γδ T cell-based cancer therapies.

P21

B cells in cutaneous inflammation

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INTRODUCTION: The role of lesional B cells in inflammation is poorly understood. Only recently, we and others showed that lesional B cells are crucial to maintain the anti-tumor immune response in melanoma. In dermatology, lesional B and plasma cells are commonly associated with chronic inflammation and are frequently observed in chronic wounds, cutaneous lupus erythematodes and hidradenitis suppurativa. At the same time, other chronic inflammatory diseases such as alopecia areata or psoriasis display minimal or complete absence of B cells, even if lesions persist for many years. This indicates that only specific types of inflammation lead to the recruitment of lesional B cells.

METHODS: In order to characterise the role of B cells in cutaneous inflammation, we created a large collection of public scRNAseq dataset of various dermatoses and integrated them with inhouse data of previous and ongoing projects. This dataset currently already spans 20 diseases, from 228 samples, with a total of over 1.1 million cells. We then subclustered the nearly 45,000 B cells to arrive at a detailed phenotypic characterisation.

RESULTS: In an initial analysis, we already observed first marked differences between B cell-rich and B cell-poor conditions. Plasmacytoid dendritic cells, for example, seem to be tightly linked to the presence of B cells in certain diseases. Moreover, due to the large number of B cells in this dataset we were able to identify 17 discrete subtypes of B cells. Comparing this composition of B cell phenotypes in B cell rich diseases, we observed marked differences between the investigated diseases.

CONCLUSION: Our findings demonstrate the first distinct differences between B cell-rich and B cell-poor inflammation. This large dataset collection will enable us to conduct an extensive analysis of the role of B cells in cutaneous inflammation.

P22

Increased circulating IL-17+ TRM are associated with squamous cell carcinoma in patients with recessive dystrophic epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is the severe form of Epidermolysis bullosa (EB), which involves a mutation in the COL7A1 gene, which encodes type VII collagen (C7). The skin of patients with Epidermolysis bullosa (EB) is highly fragile due to inherited mutations in genes that encode proteins with roles in keratinocyte integrity and cellular adhesion. In these patients the wounds are commonly infected with opportunistic pathogens and often become chronic. The chronicity of the wound microenvironment seems to provide an ideal environment for the formation of highly aggressive cutaneous squamous cell carcinomas (SCC), which are one of the most feared complications in RDEB patients. We hypothesize that chronic inflammation promotes RDEB SCC tumors. Indeed, the levels of multiple cytokines (e.g., IL-17A, IL-21, IL-22, TNF-α, GM-CSF) were significantly increased in the plasma and skin blister fluids of RDEB patients compared to healthy donors, suggesting a mixed inflammatory response. Despite this diverse inflammatory profile, only IL-17A-producing skin tropic CD103+CLA+CD4+ circulating tissue-resident memory T cells were significantly increased in RDEB patients with SCC compared to RDEB patients without SCC or healthy donors but not other Th-subsets. To further study the impact of the tumor microenvironment on immune cells we developed a novel 3D organotypic skin/tumor culture with CD4 T cells and observed elevated IL-17A production within the SCC tissue suggesting the crosstalk between tumor cells and immune cells, which is consistent with the hypothesis that the tumor microenvironment drives T cell dysfunction which in turn promotes the aggressive nature and growth of RDEB SCC. To mechanistically test this hypothesis and elucidate cellular communication between structural cells and immune cells we are using 3D cultures and RDEB SCC xenograft mouse models.

P23

The impact of re-expression of type-XVII collagen on DNA methylation

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Introduction: The differential expression of COL17A1, a major component of hemidesmosomes, has not only been shown to be a key factor in conferring stability to the dermo-epidermal junction of the skin, but also to impact stem cell competition during skin homeostasis and aging. Here, we aimed to elucidate mechanisms linked to the differential expression of COL17A1, with a specific focus on epigenetics.

Methods: DNA methylation analyses of C17-deficient keratinocytes that were corrected or not using the CRISPR-Cas technology, was performed using the Infinium MethylationEPIC BeadChip array. We analzyed genome-wide DNA methylation data using the minfi workflow in statistical software R. Quality control steps included assessment of signal intensity, detection of P-values, and removal of low-quality probes and samples. Post filtering, data normalization was performed using established methods such as quantile normalization. To identify differentially methylated CpG sites (DMCs), we employed the limma framework, adjusting for relevant covariates identified through interpretation of dimension reduction plots such as principal component analysis. Finally, we utilized epigenetic clocks to assess the impact of COL17A1 correction on aging-related processes.

Results: We found a total of 989 DMCs with $p<0.01$ that could be assigned to 1,112 genes. Functional analysis revealed pathways like hedgehog signalling to be associated to differentially methylated gene. Of interest, a change in methylation profiles also led to a difference in phenotypic age predictions, using epigenetic clocks developed by the Horvath lab.

Conclusion: Our data give first hints on the impact of C17 expression on DNA methylation patterns in human keratinocytes.

P24

Unraveling the role of a CD64+ DC population in melanoma

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Our recent research has identified a distinctive cDC2 phenotype in transplantable melanoma mouse models, characterized by FcgammaRI/CD64 expression - typically associated with monocytes and macrophages rather than DC. Notably, CD64+DC are abundant in transplantable tumors and tumor-draining lymph nodes (tdLN) but rarely found in lymph nodes (LN) of tumor-free mice. These CD64+DC express the DC activation marker CD40, suggesting their involvement in initiating effector T cell responses, as well as the co-inhibitory receptors PD-L1 and PD-L2, indicating a potential regulatory role in tumor immunity. Interestingly, not all CD64+DC express the DC-lineage defining marker Zbtb46, as shown by a Zbtb46-GFP DC-reporter mouse strain, suggesting that CD64+DC might originate from both pre-DC and monocytes. Our project aims to understand the developmental origin and functional role of CD64+DC in tumor immunity.

We examined the developmental fate of CD64+DC by injecting FLT3L, which drives pre-DC differentiation and proliferation of DC in vivo. Our investigations showed that CD64+DC, like other cDC subtypes, expand in both tumors and tdLN, indicating their dependency on the FLT3-FLT3L axis.

Next, we investigated the functional role of CD64+DC in antigen uptake by using a D4M cell line expressing ZsGreen. We observed that CD64+DC have a superior ability to take up ZsGreen+ tumor material and subsequently transport tumor-derived antigens to the tdLN. Indeed, CD64 expression on DC correlated with their capacity for antigen uptake and trafficking, as most ZsGreen+ DC in the tdLN were CD64+DC. Currently, we are studying the ability of CD64+DC to present tumor antigens to T cells.

Overall, our research aims to uncover the role of CD64+DC in cancer immunity, exploring whether they contribute to anti-tumor T cell responses or play a regulatory role. The findings will provide insights into DC heterogeneity and their relevance to tumor immunity, potentially leading to novel immunotherapeutic approaches for melanoma treatment.

P25

The impact of the tumor micromilieu on γδ T cell function in squamous cell carcinoma in Epidermolysis bullosa

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Epidermolysis bullosa (EB) is an inherited skin disorder, characterized by mucocutaneous fragility and blister formation upon minimal trauma. Patients who suffer from the severe form, recessive dystrophic EB (RDEB), often develop highly aggressive cutaneous squamous cell carcinomas (SCC). SCC in RDEB patients has a higher morbidity and mortality compared to SCC in patients without RDEB, although they share similar driver mutations. The patho-mechanisms are still largely unknown and currently there is no effective therapy available. Therefore, we analyzed whether the aggressive nature of SCC in RDEB patients is associated with a dysfunction in tumor immune surveillance. Gamma Delta (γδ)T cells display anti-tumor functions and the presence of tumor infiltrating γδ T cells was the most significant favorable prognostic immune population among 39 human cancer types. We discovered that the fraction of IFN-γ producing cells was reduced among circulating and tumor infiltrating γδ T cells isolated from RDEB patients with SCC. Based on these results, we hypothesize that γδ T cells are dysfunctional, and that this dysfunction is mechanistically linked to the formation of SCC. Whereas γδ T cell function has been studied in various skin cancer entities, their role in regulating the growth of the uniquely aggressive SCC in RDEB patients has not been investigated. To fill this knowledge gap, we mechanistically dissect how the SCC tumor microenvironment modulates the function of γδ T cells in RDEB patients. We utilize our innovative and unique in vitro 3D organotypic skin and in vivo tumor xenografting models to elucidate the cellular communication between γδ T cells and tumor cells. This study will provide comprehensive and mechanistic insights on the anti-tumor function of γδ T cells in RDEB patients with SCC. Furthermore, our results will contribute to the development of effective immuno-therapies against the highly aggressive SCC in RDEB patients.

P26

HDACs regulate skin fibroblast lineage commitment and fibrosis

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Introduction: Dermal fibroblasts are heterogeneous. During development, dermal fibroblast progenitors differentiate into different fibroblast lineages which have distinct roles in skin homeostasis, cutaneous wound repair and skin fibrosis.
Epigenetic regulation, particularly histone acetylation, is crucial for cell differentiation, but its specific role in fibroblast lineage commitment remains largely unexplored[2]. Histone deacetylases (HDACs) modulate gene expression and have been implicated in fibrosis across different organs. However, whether HDACs control dermal fibroblast fate determination and are, thus, key players in fibrotic skin diseases is unknown.

Method: In this study, we aimed to elucidate the function of HDACs in regulating fibroblast lineage commitment and skin fibrosis. HDAC inhibitors (HDACi) were injected into pregnant mice to investigate its effect on fibroblast lineage commitment during development. In addition, primary dermal fibroblasts were treated with HDACi to examine their effect on TGFb-stimulated myofibroblast differentiation and activation, and on insulin-induced adipocyte differentiation. Furthermore, HDAC expression was analyzed in a mouse model for skin fibrosis and in human fibrosis biopsies.

Result: Intriguingly, HDAC1/2 were highly expressed in both human and mouse skin fibrosis compared to healthy skin.
HDAC inhibitors promoted adipogenic differentiation and blocked TGFβ1-mediated fibroblast activation and differentiation into myofibroblasts in vitro. In vivo application of HDACi showed that blocking histone deacetylation affects dermal fibroblast lineage commitment during development.

Conclusion: Our findings indicate that HDAC inhibition can change both fibroblast lineage commitment during skin development, and the differentiation of fibroblasts into myofibroblasts or adipocytes. These results highlight the importance of HDACs in fibroblast fate determination and their potential role in skin fibrosis. Targeting HDACs may offer a novel therapeutic strategy for treating fibrotic skin diseases.

P27

TGF-ß induced changes in gene signatures related to pseudosyndactilies in epidermolysis bullosa

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Introduction: Recessive dystrophic epidermolysis bullosa (RDEB) is a monogenic skin condition marked by severe skin fragility and severe secondary complications. Previous studies highlighted transforming growth factor beta 1 (TGF-ß1) signaling to be a key player in shaping the RDEB phenotype. This study aimed to replicate conditions resembling severely affected skin by continuously stimulating RDEB-fibroblasts with TGF-β1 and analyze the resulting molecular changes to identify potential modifiers influencing disease expressivity.

Methods: To identify differential gene methylation events, we employed the Infinium Methylation EPIC bead chip array (Illumina) using the HD Assay Methylation Protocol (15019519 v01) and an Illumina NextSeq 550Dx instrument for array scanning. For identifying differentially expressed genes, we isolated DNA/RNA from RDEB-fibroblasts treated with TGF-ß1 for four weeks and performed RNA-sequencing with a NovaSeq instrument. Genes that were hypomethylated and overexpressed, or vice versa, were nominated as candidate genes for further analysis using molecular biology techniques.

Results: A total of 23 genes were found to be deregulated regarding their expression and/or methylation after long-term treatment of RDEB-fibroblasts with TGF-ß1. Of those, eleven met the criteria of being hypomethylated/overexpressed or hypermethylated/downregulated. Molecular signature enrichment analysis highlighted syndactilies among most deregulated pathways, with gremlin-1 (GREM1), a well-known player in fibrosis, as a major contributor. Confirmation of GREM1 deregulation was done by qPCR and Western blot analysis.

Conclusion: Pseudosyndactilies represent a massive burden for RDEB patients due to loss of hand functionality. To date, the pathomechanism underlying this phenomenon has not been fully elucidated. Although initial therapeutic approaches can be found in clinical research, the only current treatment option is surgical separation of the fingers, offering only short-term success. Therefore a better understanding of the mechanisms underlying EB-associated syndactilies is crucial to potentially find ways to improve treatment or prevent fusion of digits in the future.

P28

A diverse pool of oral mucosa-resident memory T cells protects against viral infection at the site of entry

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Introduction: A long-lasting protection against viral infections, including SARS-CoV-2, is based on the generation and retention of memory T cell populations within the affected tissues. As one of the first sites of contact with infectious aerosols, the oral mucosa and its specific T cell subpopulations is of interest for the evaluation of adaptive cellular immune responses to vaccinations or natural infections.

Methods: We performed droplet-based single cell RNA sequencing and spatial transcriptional profiling (Visium, 10X-Genomics) on blood and oral mucosa samples from healthy, SARS-CoV2 recovered individuals one month after infection. Cell types were annotated based on widely accepted marker genes and Python package CellTypist. Differential gene expression and TCR receptor analysis were performed using the Python toolkits SCANPY and SCIRPY.

Results: We found that the distinct distribution of T cell subpopulations in the blood and oral mucosa depends on virus-specificity. The majority of SARS-CoV2-specific T cells in blood samples were central memory T helper cells (TCM) and, in oral mucosa samples, cytotoxic tissue-resident memory T cells (TRM). The differential gene expression between SARS-CoV2-specific T cells and other virus-specific T cells showed that recently generated SARS-CoV2-specific TRMs expressed increased genes involved in migration, early tissue residency and regulation of TCR activation. Cell-cell communication analyses demonstrated strong interactive profiles of virus-specific TRMs, in particular chemokine and integrin-mediated incoming signaling originating from fibroblasts, potentially retaining TRM in the mucosa.

Conclusion: Our data shows a diverse distribution of T cell subpopulations and their respective TCR-specificity in healthy, adult oral mucosa shortly after SARS-CoV2infection. In addition, our data indicate communicative networks led by oral mucosa fibroblasts to support TRM survival and activation within the tissue. This ongoing project may contribute to further understanding of T cell responses at effector sites following viral infection.

P29

A striking case of a single patient presenting with mycosis fungoides- and lymphomatoid papulosis-like lesions analyzed using single-cell RNA sequencing

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Background: As the most frequent subtype of cutaneous T-cell lymphomas (CTCLs), Mycosis fungoides (MF) affects around 60% of cases.[1] [2] Lymphomatoid papulosis in contrast is a benign CD30-positive lymphoproliferative disorder that is no longer considered a true lymphoma.[3] This case-report presents a patient diagnosed with mycosis fungoides who concomitantly exhibits lym-phomatoid papulosis (LyP) like-lesions. FACS analysis indicated blood involvement due to which a stem cell transplantation was already planned.

Methods: To investigate the cellular and molecular characteristics of these distinct lesions, we performed single-cell RNA sequencing (scRNA-seq) coupled with single-cell TCR sequencing on skin biopsy samples from both MF- and LyP-like lesions, as well as a peripheral blood sample.

Results: Our analysis revealed distinct transcriptional profiles between the skin biopsy samples and the blood sample of the same donor. Surprisingly, we were able to identify the same T-cell clone in the clinically distinct cutaneous lesions. However, we were not able to detect this clone in the blood sample. Here, we found that the expanded clone detected in the FACS analysis seemed to be part of the patient's anti-tumour immune response.

Conclusion: These findings highlight the complex nature of CTCLs and its possible clinical overlap with LyP – yet with important implications for the clinical management of these patients. This case emphasizes the value of scRNA-seq in unraveling the wide range of clinical and histopathologi-cal presentations of CTCL.

References:
1. Latzka, J. et al. EORTC consensus recommendations for the treatment of mycosis fungoides/Sézary syndrome –Update 2023. Eur. J. Cancer (2023).
2. Rindler, K. et al. Single-cell RNA sequencing reveals markers of disease progression in pri-mary cutaneous T-cell lymphoma. Mol. Cancer (2021).
3. Kempf, W. et al. Classifications of cutaneous lymphomas and lymphoproliferative disorders: An update from the EORTC cutaneous lymphoma histopathology group. J. Eur. Acad. Der-matol. Venereol.(2024).

P30

Development of an immunocompetent 3D bioprinted melanoma-on-chip model

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In skin cancer, DC play a central role in anti-tumor immunity. Unveiling their role in cancer development, progression, and potential treatments is crucial in improving patient survival. However, tumor immunology research heavily relies on rodent models and translating these findings to the patient situation is difficult. Establishing a 3D bioprinted immunocompetent melanoma-on-chip model will enable detailed investigations of cellular interactions in the tumor microenvironment.

The first steps in establishing this 3D melanoma model involved testing various chip designs. To mimic the dermis, human foreskin fibroblasts were embedded into bioink consisting of gelatin-methacrylate and collagen. On top of the dermis a thin layer of human keratinocytes was printed. Keratinocyte differentiation was confirmed by western blot analysis of cytokeratin 1 and 10 and histology of FFPE-material was performed. Depending on chip design differentiation of multi-layered epidermis on top of the fibroblast containing dermis could be observed.

As a next step, we tested how dendritic cells (DC) can be incorporated into the dermal compartment. Using flow cytometry analysis, we observed that moDC remained viable in the hydrogels, exhibited an immature phenotype when analyzed by flow cytometry, and could be activated within the hydrogels by the addition of a maturation stimulus. However, to reflect the complexity of human DC populations, protocols were established to differentiate cDC1, cDC2 and pDC from CD34+ hematopoietic stem cells derived from human bone marrow. In future, these in vitro differentiated DC will be incorporated into 3D bioprinted melanoma-on-chip model to investigate the interactions between melanoma cells and DC subtypes.

P31

Lipid imbalance as risk factor in sarcoidosis

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Sarcoidosis is a multisystemic disease characterized by the formation of non-caseating granulomas. In granuloma-associated macrophages, several metabolic pathways, including cholesterol synthesis, are upregulated, contributing to the chronic inflammatory process. Clinically, abnormalities in blood lipid profiles of sarcoidosis patients have been observed, and statins have been shown to improve disease outcome in a mouse model and in humans. Therefore, we aim to provide insights in the prevalence of dyslipidemia in sarcoidosis patients.

We retrospectively evaluated the blood lipid profiles of 40 treatment-naïve sarcoidosis patients without statin treatment (27 females; 13 males) with various organ manifestations, including the skin and lungs, assessed at the Medical University of Vienna / Vienna General Hospital. These profiles were compared to those of the general Austrian population, and to clinically recommended threshold.

55.0% (n=22) of patients had cholesterol levels above the clinical upper limit of 200mg/dl, and 20% (n=8) of patients exhibited very high cholesterol levels (>239mg/dl). 37.5% of patients (n=15) had cholesterol levels exceeding the Austrian population average.
Elevated LDL cholesterol (>100mg/dl) was observed in 62.5% (n=25) of patients, and 15.0% (n=6) had very high levels (>160mg/dl). 32.5% of patients (n=13) exceeded the Austrian LDL average.
HDL cholesterol was below the lower threshold (females <55mg/dl; males: <45mg/dl) in 45.0% (n=18) of patients, and 37.5% (n=15) had HDL levels below the Austrian average.
Triglycerides were elevated in 22.5% (n=9) of patients (150-199 mg/dl), while 15.0% (n=6) showed very high levels (>200mg/dl). Overall, 52.5% (n=21) had triglyceride levels exceeding the Austrian population average.

To summarize, a significant number of sarcoidosis patients exhibit a dysregulated lipid profile, with more than one third having lipid values above the Austrian population average. This highlights the need for routine dyslipidemia screening, as lifestyle changes or pharmacological interventions aimed at restoring the metabolic balance may help mitigate systemic granulomatous inflammation.

P32

Senescence and SASP: Relationship between GDF15 and Skin Aging

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Cellular senescence is a well-regulated biological phenomenon that represents a cell's state of irreversible growth. A double-edged sword, senescence has both beneficial and harmful effects, influencing the skin through age-related changes such as impacting skin structure and function, and playing a role in many skin-associated diseases (1, 2). One hallmark of cellular senescence is the senescence-associated secretory phenotype (SASP), which involves a complex secretion of pro-inflammatory cytokines, chemokines, and extracellular vesicles (EVs) acting to influence immune surveillance and intercellular communication (3, 4). One known member of the SASP, Growth differentiation factor-15 (GDF15), is a stress-response mitokine. GDF15 is ubiquitously expressed, having a wide range of functions extending from regulating appetite to serving as a biomarker for aging, and importantly, having a protective role during stress and aging (5).

By modulating GDF15 expression in human dermal fibroblasts, we unravel its impact on mitochondrial health by investigating the fusion/fission dynamics and mitophagy. We are also actively uncovering the changes in SASP and their influence on local fibroblasts, keratinocytes, and the immune response. We have demonstrated that GDF15-deficient fibroblasts exhibit mitochondrial dysfunction and undergo premature replicative senescence, with distinct characteristics such as increased cell surface area and senescence-associated beta-galactosidase detection, as well as decreased replication and an abnormally fused mitochondrial network. GDF15-depleted fibroblasts have a higher EV secretion rate with unique cargo, which, within 3D skin models, induces epidermal thinning in neighboring keratinocytes (7).
Understanding the roles of GDF15 and the SASP is essential for deciphering the complexities of aging and cellular senescence. GDF15's significance in these processes highlights its potential as a therapeutic target, in particular, in the evolving field of senotherapeutics. Increased knowledge of GDF15 and SASP mechanisms could lead to innovative strategies for addressing age-related diseases and improving skin health.

P33

HDAC1 as a regulator of CD4+ T cell maintenance in skin autoimmunity

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The skin constitutes the first line of defense against pathogens and toxins derived from the environment. It mounts host-protective responses while maintaining immune homeostasis. These functions are fulfilled by immune cells, including CD4+ T cells. Histone deacetylases (HDACs) regulate the acetylation status of histones and non-histone proteins by removing acetylation marks on lysine residues. Hence, HDACs control diverse biological processes, such as differentiation and function of CD4+ T cells. However, the exact role of HDAC1 in CD4+ T cells, specifically in skin autoimmune diseases, is not well understood.

To assess the role of HDAC1 in the regulation of cutaneous CD4+ T cell differentiation and function, we utilized a well-established mouse model of experimental skin autoimmune disease (K5/TGO) in which ovalbumin (Ova) is expressed by keratinocytes in a tetracycline-dependent manner. We adoptively transferred WT or HDAC1-deficient (HDAC1-cKO) naïve Ova-specific CD4+ OTII T cells in K5/TGO. Transfer of HDAC1-cKO OTII T cells elicited increased inflammation in response to Ova-expression in the skin compared to WT OTII T cells. This correlated with a decreased fraction of peripherally induced Foxp3+ Treg in HDAC1-cKO OTII recovered from K5/TGO recipients and with increased numbers of HDAC1-cKO T cells in the skin-draining lymph nodes (sdLNs).

Preliminary analysis of scRNA-sequencing data from these mice indicated that HDAC1 differentially regulates genes involved in immune regulation and T cell maintenance, which may regulate T cell differentiation and persistence in skin autoimmunity.

P34

A psoriasis-like CD4+ T cell-mediated mouse model of skin autoimmunity

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As a major barrier organ of the body, the skin forms the first line of defense against invading pathogens and environmental threats, whereby an interplay of effector T (Teff) and regulatory T (Treg) cells governs tissue homeostasis. Dysregulation of function and differentiation of cutaneous CD4+ T cells is associated with autoimmune diseases such as psoriasis, which is characterized by chronic skin inflammation and affects 2-4% of the global population. Available mouse models of psoriasis do not replicate the complex patho-mechanisms. Particularly, current models are not driven by self-antigen specific CD4+ T cells and a mouse model that resembles human psoriasis as a CD4+ T cell-mediated skin autoimmune disease is required to perform mechanistic studies of the inflammatory processes.

We characterized a mouse model of CD4+ T cell-mediated skin autoimmunity (designated K5/TGO) that features doxycycline-inducible expression of the self-antigen ovalbumin (OVA) in the epidermis. Adoptively transferred naïve OVA-specific OTII CD4+ T cells induced cutaneous autoimmune disease characterized by skin wounds, scaling, erythema, alopecia and eye inflammation. Histologically murine skin lesions resembled lesional skin from psoriatic patients including epidermal thickening or dermal inflammatory infiltrates. The autoimmune disease is mainly driven by Th1 and Th17 cells, and single cell RNA-sequencing revealed gene signatures in skin cells that resembled human psoriasis. With this data, we aim to provide a novel T cell driven mouse model to study psoriasis as a basis to explore new treatment options of this skin autoimmune disease.

P35

Effects of immunomodulatory CAFs on macrophage polarization in melanoma

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Melanoma remains one of the most lethal cancers worldwide, with rapidly increasing incidence and significant therapeutic challenges. Despite advances in immunotherapy, a substantial proportion of patients exhibit resistance, largely due to the complex interplay within the tumor microenvironment (TME). Central to this resistance is the presence of cancer-associated fibroblasts (CAFs), which are emerging as key regulators of immune evasion and tumor progression. Previous findings from our lab have identified distinct CAF subsets in various skin cancers, including basal cell carcinoma and squamous cell carcinoma, with immunomodulatory CAFs (iCAFs) notably enriched in aggressive melanomas. We were also able to show that in vitro induced iCAFs can activate CD4+ and CD8+ T cells and stimulate their proliferation. We aim to continue exploring the interactions between iCAFs and various immune system components, particularly innate immune cells such as macrophages. To explore this, we treat M0 macrophages derived from PBMCs with conditioned media from healthy dermal fibroblasts, iCAFs, and primary melanoma CAFs, assessing their polarization into M1 or M2 phenotypes using flow cytometry. Through direct co-culture of M0 macrophages with fibroblasts or iCAFs, we expect even more pronounced polarization shifts. Additionally, iCAF-educated macrophages are introduced to primary melanoma cell lines to evaluate their impact on tumor cell proliferation. Our preliminary data suggest that iCAFs skew macrophage polarization toward an M2-like state, as indicated by an increase in CD163-expressing cells. M2 macrophages are known to be pro-tumorigenic, so we anticipate they will promote increased melanoma cell proliferation. In summary, our research offers valuable insights into how iCAFs influence immune responses in melanoma, particularly through macrophage modulation. The findings may pave the way for more targeted therapies, improving both survival rates and quality of life for melanoma patients.

P36

Investigation of Predictive Markers for Psoriasis Arthritis

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Introduction: Psoriasis is often diagnosed effortlessly, and modern treatments are effective in controlling the cutaneous disease. However, psoriatic arthritis (PsA) is primarily diagnosed by exclusion, leading to delayed preventative therapy, high disease burden, and potentially irreversible joint damage. Due to a limited understanding of its pathogenesis, specific approaches for PsA remain underdeveloped. This study aims to identify predictive diagnostic markers for PsA in patients with cutaneous psoriasis to enable earlier treatment and prevention.

Methods: This prospective study includes patients with active cutaneous psoriasis vulgaris and no clinical signs of PsA. Patients were recruited from the Psoriasis outpatient clinic of the Department of Dermatology, and lesional punch biopsies were obtained. All patients underwent high-resolution ultrasound at the Department of Rheumatology to detect subclinical PsA. RNA sequencing (RNA-seq) was performed on all skin samples. Fresh frozen skin sections were prepared using a cryotome, and RNA was extracted with Trizol. Sequencing was completed at the Biomedical Sequencing Facility at CeMM.

Results: 76 patients were recruited, and skin samples were analyzed via bulk RNA-seq. Despite good RNA quality (photometric values: 1.7–2.0; RNA concentrations: 30–300 µg/µL), the RNA Integrity Numbers (RIN) were below 10, likely due to a freezer malfunction affecting sample storage. Bulk RNA sequencing was conducted using QuantSeq to successfully analyze all 76 samples. Ultrasound identified subclinical PsA in 12 patients. No significant differences in cutaneous lesions between PsA and non-PsA patients were observed at the RNA level.

Conclusion: Initial findings suggest that PsA cannot be differentiated from cutaneous psoriasis using RNA expression profiles. Future analyses will focus on protein-level differences.

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Assessment of ‘omics-based biases in pathway analyses using ReactomeGSA

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Introduction: Next Generation Sequencing (NGS) technologies, such as Microarray and RNA sequencing are key technologies for biomedical research. Pathway analyses are one of the most widely used methods to analyze such data. Yet, it is currently unknown whether there are systematic differences between the used ‘omics technology and the observed regulated pathways.This study investigates potential biases across these technologies by analyzing over 1,500 datasets from GREIN and Expression Atlas using ReactomeGSA.

Methods: Datasets from these public repositories for ‘omics data were analyzed via ReactomeGSA using two pathway algorithms, PADOG and Camera, to identify pathway expression patterns. PADOG down-weights the influence of genes shared across multiple pathways, improving specificity, while Camera adjusts for gene-gene correlations within pathways, providing more accurate pathway enrichment. The comparison focused on how different technologies, combined with these algorithms, influenced pathway detection.

Results: Preliminary analysis suggests variation in the number of pathways detected across different technologies and algorithms. PADOG and Camera showed differences in the number and type of significant pathways identified, with PADOG generally detecting more pathways, potentially reflecting increased sensitivity. Variations were also observed between technologies, indicating potential biases related to specific platform characteristics. Further analysis is ongoing to determine the extent of these differences and their implications for pathway enrichment.

Conclusions: Both the choice of technology platform and analysis algorithm influence pathway analysis results, with each method having its own strengths and limitations. PADOG appears to offer higher sensitivity, while Camera may provide more conservative estimates, highlighting more robust pathways. These initial findings suggest that cross-validation using multiple platforms and algorithms is essential to ensure reliable biological interpretations.

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Primary cutaneous follicle center lymphoma spans yet unrecognised subtypes including polyclonal reactions

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Introduction: Primary cutaneous B-cell lymphomas (PCBCL) are a subset of extranodal non-Hodgkin lymphomas, categorized into three main subtypes: primary cutaneous follicle centre lymphoma (PCFCL), primary cutaneous marginal zone lymphoma/lymphoproliferative disease (MZLPD), and diffuse large B-cell lymphoma-leg type (DLBCL-LT). While MZLPD is generally considered a lymphoproliferative disorder due to its indolent nature, PCFCL, despite its similarly slow progression, is still classified as a lymphoma that necessitates full staging and ongoing follow-up to monitor for systemic disease.

Methods: Lesional punch biopsies were collected from 11 patients with histologically confirmed PCFCL. Single-cell RNA sequencing (scRNA-seq) combined with B-cell receptor sequencing was used to profile the phenotypes of the B cells within the lesions. After Mutual Nearest Neighbors (MNN) samples integration and cell annotation, differentially expressed genes analysis and Cell-to-cell communication analysis were conducted.

Results: Interestingly, despite profiling several thousand B cells per sample, only five PCFCL samples displayed a clearly expanded B-cell clone. Moreover, the observed clonal expansion was exclusively composed of germinal center B cells. This finding was unexpected, as current B-cell biology suggests that these cells should differentiate into memory or plasma cells.

Conclusions: Our findings reveal previously unrecognized heterogeneity within PCFCL, particularly in relation to clonal expansion. This suggests that PCFCL is a poorly understood disease and may include distinct, yet unrecognized, subtypes.

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Itchy and scratchy: adverse events induced by targeted EGFR-cancer therapy

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While anti-cancer therapies targeting EGFR and its downstream signalling cascade have been increasingly utilised in preventing tumour growth, their adverse events are dominated by cutaneous conditions, such as pruritus, folliculitis, and bacterial super-infections.

Our lab introduced several mouse models to study these adverse effects: EGFRΔep mice, with a keratinocyte-specific deletion of EGFR, displayed extensive skin inflammation, skin barrier, and hair follicle defects. We could ameliorate these cutaneous conditions through transgenic overexpression of K5-SOS, which re-activated the ERK signalling cascade independent of EGFR.

To check if the EGFRΔep models the EGFR-inhibitor treatment provoked itch, we analysed the frequency and length of scratch / groom events in 3-month-old+ mice. We observed a significant increase in all of the scratching / grooming parameters as compared to WT control animals.

Interestingly, the pruritic behaviour was independent of mast cells and bacterial infection in our model. However, the EGFRΔep K5-SOS mice scratched significantly less, while still having the comparable high grooming rates of the EGFRΔep mice. This indicates a persistent hypersensitive skin. Additionally, we found the pathogenic Staphylococcus aureus colonisation only in the facial skin regions susceptible to grooming. These areas were also prone to excessive skin barrier defects and eczema formation.

We therefore hypothesise that prolonged inhibition of cutaneous EGFR leads to hypersensitive skin, which is prone to the itch-scratch cycle, and that this facilitates the spread and pathogenicity of Staphylococcus aureus.
Taken together, our study aims at deciphering the sequence of events in atopic skin conditions and at developing novel mechanism-based therapeutic strategies for patients suffering from pruritis.

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First insights into the EB House Biobank: On the way towards an innovative large-scale analysis of clinical routine data

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Introduction: The EB House Biobank (EBB) at the Department of Dermatology in Salzburg is a specialized repository that collects and stores biological samples from people with epidermolysis bullosa (EB) and healthy volunteers, along with medical and genetic data. For the first time, we have conducted an analysis of key characteristics of the entire EBB.

Methods: Samples are taken upon written informed consent of the patient and/or their legal guardians in accordance with the guidelines of the University Hospital Salzburg and the Declaration of Helsinki (Ethics committee vote numbers: 415-E/2118/9- 2017). Adherence to standard operating procedures allows quality-assured optimization of sample preservation. Data analyses are based on descriptive statistics.

Results: Between 2017 and 2024, a total number of 1599 samples from 393 individuals have been included in the Biobank, with an increase from 54 samples in 2017 to 454 samples in 2024. The median age of individuals was 37 years (interquartile range 15 – 65 years; 110 (28%) missing values), and 151 persons were women (38%; 68 (17%) missing values). Most samples were swabs (591; 38%), followed by blood (460; 30%) and tissue (219; 14%) samples (missing data for 45 samples (3%)). The majority of samples were taken from patients with severe recessive dystrophic EB (703; 45%), junctional EB (166; 10%), and other subtypes of recessive dystrophic EB (153; 10%), complemented by 379 samples (24%) from healthy donors.

Conclusions: The EBB provides a wealth of data quantitatively and qualitatively, covering a broad range of sample and EB types. In order to fully unlock the potential of the EBB, in particular more standardized and structured clinical data are needed. As a next step, we will analyze associations between individual characteristics and biological / genetic data, thus supporting a better understanding of EB which paves the way for innovative therapeutic and diagnostic approaches.

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Conservation and substitution of hair keratins during the evolution of cornified skin appendages

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Hair keratins are the main protein components of human hair and nails. Recently, we have shown that homologs of hair keratins build the cornified claws of clawed frogs. To gain further insights into the evolution of skin appendages, we investigated cornified epidermal spines, which develop on different body sites of phylogenetically diverse frogs.

The analysis of transcriptome data showed that the expression of hair keratin homologs correlates with the development of nuptial spines in the Emei moustache toad (Leptobrachium boringii). In the clawed frogs, Xenopus laevis and X. tropicalis, cornified spines were detected histologically on the skin of forelimbs. RT-PCR analysis and proteomic analysis showed that the type II hair keratin homolog krt59 is expressed in the skin containing these spines. By contrast, the type I hair keratin homolog of amphibians, krt34, is absent and apparently replaced by other type I keratins that are upregulated in the cornified epidermal spines of Xenopus frogs.

These data suggest that the contribution of hair keratin homologs to the development of cornified skin appendages is at least partially conserved across terrestrial vertebrates. In addition to a previously reported substitution of one type of hair keratins in the claws of birds, we present evidence for a similar substitution in cornified skin appendages of Xenopus frogs, indicating recurrent molecular diversification of an ancestral cornification program.

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scRNA-seq based characterisation of Alopecia areata questions the role of T cells in the clinical hair loss

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Introduction: Alopecia areata (AA) is an autoimmune disorder characterized by non-scarring hair loss, which can manifest in localized patches or progress to complete loss of scalp and body hair. The pathogenesis involves the immune system mistakenly targeting hair follicles by collapse of the hair follicle's immune privilege, with T-cell mediated inflammation playing a key role. Though the exact etiology remains unclear, genetic predisposition and environmental triggers are thought to contribute. AA exhibits variable disease courses, with spontaneous remissions and relapses, necessitating tailored approaches in clinical management and ongoing investigation into potential therapeutic targets.

Methods: We obtained 28 biopsies of patients previously diagnosed with AA. 24 of which have been processed immediately after sample collection, 4 after freezing, using 10x scRNAseq protocols for sample preparation and the 10x Chromium Controller for scRNA-seq sequencing, followed by bioinformatic analysis.

Results: For this study, we investigated samples from patients with three types of AA: alopecia areata totalis, alopecia areata universalis, and patch-type alopecia areata in different treatment stages and of lesional and non-lesional states. First results show that, surprisingly, and contrary to the current opinion, no difference in T cell infiltrate is seen between lesional and non-lesional skin samples. Furthermore, hair follicle-associated keratinocytes and fibroblasts increased in the resolving sample.

Conclusion: Our data shows that previously expected differences in T cell behavior between lesional and non-lesional AA cannot be observed in our scRNA-seq data. Additionally, as expected, we showed that there are specific differences in cell composition in lesional and non-lesional samples. This highlights that the pathogenesis of AA and its subtypes are not yet sufficiently understood, and require further investigation.

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TRIM28 controls epidermal homeostasis by regulating p53 activity

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Background: Tripartite motif-containing 28 (TRIM28) is a member of the TRIM protein family, some of whose members have transcription factor properties or E3-ligase enzymatic functions. TRIM28 has been shown to interact with the p53 tumor suppressor mouse double minute 2 homolog (MDM2) to promote the proteasomal degradation of p53. However, its contribution to skin homeostasis remains unknown.

Methods: We used transcriptomic analysis and immunofluorescence staining to investigate TRIM28 expression in the epidermis. siRNA-mediated gene silencing was employed to examine the role of TRIM28 in epidermal keratinocyte (KC) differentiation and the formation of the epidermal barrier in 3D skin models.

Results: To investigate gene expression patterns during the differentiation of epidermal keratinocytes, we conducted gene expression profiling of undifferentiated primary human KCs, differentiated KCs in monolayer cultures, and 3D skin models. Bioinformatics analysis identified TRIM28 as a factor specifically active in 3D skin models. Strong TRIM28 mRNA and protein expression in the epidermis, across all layers except the terminally differentiated KC layer, was detected by single-cell sequencing and immunostaining. To explore the role of TRIM28 in epidermal development and barrier formation, we performed TRIM28 knockdown in KCs and subsequently established skin models. Morphologically, TRIM28-deficient skin models appeared fully differentiated but were significantly thinner due to the induction of apoptosis in basal KCs. While TRIM28 knockdown did not affect overall protein ubiquitination, p53 ubiquitination was specifically inhibited.

Conclusion: Our findings suggest that TRIM28 E3-ligase activity in the basal layers of the epidermis plays a crucial role in regulating proper epidermal formation by modulating p53 activity.

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γδ T cell and macrophage interplay within the ER stress tumor microenvironment

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γδ T cells play a dual role in tumor immunity, with both anti-tumor and pro-tumorigenic functions, depending on how they are influenced by the tumor microenvironment. The mechanisms behind this functional contradiction are not fully understood. Endoplasmic reticulum (ER) stress, caused by the accumulation of misfolded proteins, is known to promote tumor growth and alter immune cell functions within the tumor microenvironment. When γδ T cells were exposed to conditioned media from ER-stressed cutaneous squamous cell carcinoma (cSCC) cells, they upregulated genes involved in monocyte recruitment and macrophage differentiation. Additionally, these γδ T cells released higher levels of IL-4 and IL-10, cytokines known to induce M2 macrophage polarization, a key characteristic of tumor-associated macrophages (TAMs) that support tumor growth and angiogenesis. Given that ER stress is a common feature of the tumor microenvironment, we hypothesize that ER stress alters γδ T cell function, which in turn promotes the differentiation of macrophages toward a pro-tumorigenic M2 phenotype. No studies have yet explored the interaction between γδ T cells and macrophages in the ER stress tumor microenvironment. This study aims to investigate how ER stress-conditioned γδ T cells influence macrophage differentiation and polarization toward either the M1 or M2 phenotype. The findings will provide new insights into how γδ T cells regulate macrophage anti-tumor responses under ER stress, potentially revealing new targets for cancer immunotherapy.

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Several cases of pigment induced complications

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Introduction: Recently the number of tattooed people has raised. The most common skin reactions to tattoo include medical complications such as allergic contact dermatitis, granulomatous and lichenoid reactions, and skin diseases localized on tattooed area (eczema, psoriasis, lichen, and morphea).

Methods: Patient A-37 years old female first presented with complains of itch and erythema on left shin, in same location patient had tattoo which was tattooed one year before reaction, also patient had hypothyroidism. During the first appointment locally on left shin was allergic contact dermatitis complicated with fungal infection. According to clinical simptoms Tab. Desloratadine 5 mg twice a day and topically Diflucortoloni valeras+Isoconazoli nitras was prescribed. In three weeks without improvement of therapy lesion continued to cause complains of severe itch, also because of picture of chronic allergic contact dermatitis with fibrotic tissues local i/c injections of Sol.triamcinoloni acetonidum was performed. Two weeks after first injections complains decreased, locally lesions reduced. Due to positive effect on i/c injections therapy reccomendations to continue i/c injection therapy was given to patient, but according to recieved information that patient is 6 weeks pregnant first there was a need to consult gynicologist about possibility to continue therapy with Sol.triamcinoloni acetonidum.
Patient B–36 years old female firsts presented with complains of smalt erythematose lesions on left eyebrows, a little itchy, in same location patient has done permanent make up. In dermatoscope–small 0,5 cm erythematose lesion with pigment inclusions, small neovascularization. On next appointment biopsy was performed. Histology showed in the dermis, several well-demarcated tiny epithelioid cell granulomas; perifocally and in the interstitium, lymphohistiocytic infiltration with the presence of fibroblasts, black pigment deposits of exogenous origin (negative Perls reaction), hemosiderin deposits (positive Perls reaction). Histological picture corresponds to Sarcoid granulomatous reaction to tattoo. Topical therapy with Methylprednisoloni aceponas was prescribed, in two months patient had positive reaction to local therapy.

Results and Conclusion: Complications after tattoo has many faces, but therapy possibilities should be evaluated individually.

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Dissecting the role of cancer-associated fibroblasts and extracellular matrix dynamics in skin cancer

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Cancer-associated fibroblasts (CAFs) exert significant influence on cancer initiation, progression, and response to therapy. The tumor microenvironment (TME) harbors multiple CAF subsets with tumor-promoting or -suppressing properties. Understanding CAF heterogeneity within the TME is crucial for defining their complex roles in skin cancers. We identified two CAF subtypes which modulate tumor immune surveillance. One of them, the so-called matrix-CAFs (mCAFs), encapsulate tumor nests and modulate extracellular matrix (ECM) dynamics, possibly influencing immune cell infiltration.

By employing invasion assays with various ECM compositions, alongside in situ staining, we investigated the functional role of mCAFs and ECM proteins in melanoma and non-melanoma skin cancer (basal cell and squamous cell carcinoma). Furthermore, the expression of different ECM proteins as well as the abundance of and spatial distribution mCAFs were examined in those skin cancer samples.

We observed increased invasiveness of peripheral blood mononuclear cells (PBMCs) into matrices composed of Collagen I mixed with Collagen IV, Laminin or Fibronectin compared to Collagen I alone, indicating that ECM composition affects immune cell marginalization. Additionally, the abundance of mCAFs and the expression of different ECM proteins correlated with the immune status of skin tumors (immune desert, immune excluded and inflamed cancer types). Finally, quantification of mCAFs at the tumor-stroma border and T cells within tumor nests revealed a negative correlation of mCAF enrichment and T cells within the tumor nests, indicating that mCAFs might indeed play a role in T cell exclusion.

Our findings highlight the role of ECM composition in immune cell invasiveness and suggest a role for mCAFs in modulating the ECM composition and, thus, in modulating immune cell behavior within the TME. Investigating the role of mCAFs and their ECM components will enhance our understanding of immune surveillance mechanisms and could lead to novel approaches to improve responses to immunotherapy.

P47

A case of fibroblastic tumor

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Introduction: The fibroblastic tumor is among the most common of all the soft tissue tumors involving the extremities.

Methods: 32 years old male presented with compains about itchy psoriatic generalised elements,also about neoplasm on left leg. Neoplasm first appeared 3 years ago, but in last 6 months began to actively growth, bleed and ulcerate. Locally 6 cm big neoplasm with massive neovascularization, ulceration and bleeding. To specify diagnosis biopsy was performed. Histology showed-dermatofibroma (lipid and siderotic form) with ulceration. There are no signs of malignancy in the given material. Taking into account the clinical picture, complete excision of the formation is necessary to clarify the diagnosis. MRI showed dermal and subdermal pathological neoplasm with differential diagnosis of dermatofibroma and dermatofibrosarcoma. After MRI and biopsy results patient was sent to perform full neoplasm excision. After surgery results of biopsy was received. Histology showed the tumor consists of valve-shaped cells with eosinophilic cytoplasm, cells without pleomorphism, no mitoses, in some places pronounced capillary network, lymphocytic infiltrates, multinucleated cell infiltrates, stroma with hyalinization in some places, the outer border of the formation is sharply contoured, no infiltrative growth is detected. CD31-positive, STAT6-negative. Morphological and immunohistochemical picture is consistent with deep fibrous histocytoma. In parallel patient received systematic and topical treatment against psoriasis and systemic therapy for diabetes mellites.

Results and Conclusion: Locally fibroblastic tumors can look different and important step of successful diagnostic and treatment is biopsy and requires a multidisciplinary approach for the best outcome.

P48

Cancer-associated-fibroblast subsets shape the immune landscape of non-melanoma skin cancer

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Cancer-associated fibroblasts (CAFs) are key drivers of cancer progression and therapy resistance in solid tumors. We previously identified three CAF subtypes—myofibroblast-like RGS5+ CAFs, matrix CAFs (mCAFs), and immunomodulatory CAFs (iCAFs)—in Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC). We employed molecular and spatial single-cell resolution techniques to analyze CAF diversity. Large-cohort tissue analysis revealed shifts in CAF subtype patterns with increasing malignancy. mCAFs synthesized extracellular matrix, potentially restricting T cell infiltration in lower-grade tumors by encapsulating tumor nests. In contrast, iCAFs, enriched in late-stage tumors, expressed cytokines and chemokines that facilitated immune cell recruitment and activation. This study aims to investigate how CAF subtypes interact with and influence the immune tumor microenvironment (TME) in non-melanoma skin cancers by analyzing immune cell composition, spatial distribution relative to CAF subtypes, and the expression of key immunomodulatory markers.

We developed an Imaging Mass Cytometry (IMC) antibody panel to label major myeloid and lymphoid immune cell subtypes in the TME of skin cancers. The panel included antibodies targeting exhaustion and activation markers to assess immune cell status. The Halo platform was used for image analysis, alongside Python and R programs. FlowSOM was applied for cell clustering, and Squidpy for spatial analysis to explore relationships between CAF subtypes and immune cells.

Our data suggest that SCCs harbor a higher density of immune cells than BCCs. A correlation was observed between tumor immune status (immune- desert, excluded and inflamed) and CAF composition. Tumors with inflamed immune profiles had increased iCAF presence, while mCAFs were more common in immune-excluded tumors, suggesting distinct CAF-immune interactions influencing tumor progression and therapy response.
These findings highlight the complex interactions between CAFs and the TME in non-melanoma skin cancers. Targeting specific CAF subtypes could enhance immunotherapy by reshaping the immune landscape, with broader implications for solid tumor treatment.

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Immunogenic Cell Death (ICD) and ICD-dependent Dendritic Cell Activation triggered by Extracorporeal Photopheresis in CTCL

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Immunogenic cell death (ICD) has emerged as a crucial mechanism in cancer immunotherapy, characterized by the release of damage-associated molecular patterns (DAMPs) that promote dendritic cell (DC) maturation and cytotoxic T lymphocyte (CTL) responses. Extracorporeal photopheresis (ECP) is a photochemotherapy used for cutaneous T-cell lymphoma (CTCL) treatment, involving leukapheresis, 8-methoxypsoralen (8-MOP) administration, and UVA irradiation of leukocytes. This study investigates whether ECP induces ICD in CTCL patients and healthy donors and its ability to induce DC activation. Using an in vitro ECP model on healthy peripheral blood mononuclear cells (PBMCs) and ECP-treated white blood cells (WBCs) from CTCL patients, we observed significant markers of ICD, including ATP release, HMGB1 secretion, and surface calreticulin (CALR) exposure. Our results demonstrated reduced cell viability and increased cell death in ECP-treated samples. Gene expression analysis showed significant upregulation of ICD-related genes, confirming ICD induction.

In CTCL patients, elevated CALR expression was notably higher in malignant T cells (CD26-), suggesting greater susceptibility to ICD. We further demonstrated that ECP-treated CD4+ T cells were phagocytosed by DCs, and this process was dependent on ICD signals, as blocking CALR and ATP halted phagocytosis.

Our findings reveal that ECP induces ICD in both healthy and malignant T cells, facilitating DC activation and antigen presentation. These results underscore ECP's potential in enhancing targeted immune response to malignant T cells in CTCL, offering new insights into its therapeutic mechanisms and applications in cancer immunotherapy.

P50

Analysing abnormal epidermal homeostasis in epidermolytic palmoplantar keratosis (EPPK)

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Palmoplantar keratoderma caused by the keratin-9 hotspot mutation p.N161S is characterized by debilitating diffuse hyperkeratosis on the palms and soles, often accompanied by erythema at the periphery, suggesting an associated hyperinflammatory response. To investigate the effects of the KRT9 mutation on skin function, including water balance and potential immune system involvement, we conducted a prospective study with six individuals from two generations of a family with PPK (KRT9mut PPK) compared to healthy controls (WT).Data collection included a patient questionnaire on health status and treatment of the skin disorder, clinical information, biophysical measurements of epidermal function, and the acquisition of blood and skin samples. Evaluation of the questionnaire as well as the clinical data did not show any systemic abnormality due to EPPK. Likewise immunophenotyping of blood samples showed no significant differences between patients and controls. However, stratum corneum capacitance measurements indicated reduced hydration in KRT9mut PPK individuals on the palms and soles compared to controls, while measurements on the lower arm skin showed no difference. Transepidermal water loss and skin surface pH values did not differ between the two groups independent of the site. Further analysis of the PPK phenotype using single-cell RNA sequencing (scRNA-seq) revealed increased expression of CCL2 and CXCL12 in multiple cell types, including keratinocytes and fibroblasts, while CXCL1, CCL19, and CCR7 were selectively downregulated. Altered keratin expression included upregulation of basal KRT5 and KRT14 in KRT9mut differentiated KCs, and downregulation of suprabasal KRT1, KRT10, and KRT2. Additionally, CALML5, a calcium-binding protein, was downregulated in KRT9mut differentiated KCs.

Based on these findings, we hypothesize that the keratin-9 hotspot mutation p.N161S initiates a previously unrecognized hyperinflammatory response in palmar and plantar skin of EPPK patients, which, combined with disrupted water balance, may compromise skin barrier function.

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Immune Responses against Treponema pallidum in human skin

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Syphilis is a sexually transmitted infection caused by the spirochaete bacterium Treponema pallidum (Tp). The past two decades were marked by a significant rise in primary and secondary syphilis cases, which present with various clinical manifestations including local bacterial multiplication and systemic dissemination. To date, no vaccine is available, underscoring the need for new preventive strategies. Our understanding of immune responses to Tp remains limited, primarily due to challenges in bacterial culture, historically relying on rabbit models. However, with the recent development of an in vitro Tp culture system, we can now explore Tp -host-cell interactions in human skin.
Harnessing the immune system might lead to novel syphilis interventions and we aim to elucidate Tp-specific immune responses in skin using patient samples and complex ex vivo skin and lymphoid organ models. By combining single-cell RNA sequencing and high-resolution imaging data from syphilis patient samples, we will determine gene expression changes triggered by Tp invasion in skin. Moreover, local immune responses towards Tp will be examined in human skin explants. T cell activation and polarization are assessed in a lymphoid organ model.
We detected bacterial transcripts in human skin explants at various time points following infection with live Tp, demonstrating the viability of our skin model to mimic syphilis infection. Infected skin biopsies further showed upregulation of pro-inflammatory cytokines. Utilizing a huma-derived lymphoid organoid model, we observed impaired T cell polarization upon exposure to Tp, with no significant effect on Th1, Th2 and Tregs compared to the response elicited by the pro-inflammatory bacterium Staphylococcus. aureus. Interestingly, Tp infection led to a slight increase of Th22 cells, which was not observed with S. aureus. Our findings reveal an innovative approach for studying Tp infection in human skin, offering insights into host-pathogen interactions with the potential to elucidate novel therapeutic targets for syphilis.

P52

Establishing a safe and effective LNP-mediated gene delivery system for correcting keratin mutations in the skin

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Heterozygous dominant mutations in keratin genes are implicated in debilitating skin disorders, including epidermolytic ichthyosis, pachyonychia congenita, and palmoplantar keratoderma, which present with thickened skin and increased vulnerability to infections. Our research targets the keratin-9 hotspot mutation p.N161S in palmoplantar keratoderma patients. We find a previously unreported disruption in keratinocyte differentiation, along with a heightened inflammatory response in palmar biopsies from affected individuals. To address these challenges, we have developed a CRISPR-Cas9 double-nickase approach to convert this missense variant into a nonsense mutation. This innovative strategy showed to successfully restore keratin intermediate filament stability in keratinocytes, isolated from patient´s palmar biopsies. Our carrier-free approach demonstrated a strong safety profile validated through comprehensive off-target analysis. To enhance the efficiency of our genetic editing in vivo, we aim to implement cytosine base editing for precise base changes. However, delivering these gene-editing tools to the skin remains a challenge due to the robust nature of the epidermal barrier. To address this, we propose the development of lipid nanoparticles equipped with KC-specific adhesion sequences, in combination with skin pretreatments such as microporation, to enhance penetration for topical delivery. This project aims to establish a safe and effective lipidnanoparticle-mediated gene delivery system for keratin mutation correction, thereby contributing to the advancement of therapeutic genome editing for related disorders. Our findings will pave the way for future clinical applications, offering new hope for patients affected by these severe skin conditions.

P53

Whole Slide Imaging for Keratinocyte Cancer Diagnosis: A High-Resolution Dataset for Machine Learning Research

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Abstract: In the field of computational pathology, the availability of high-quality, well-annotated datasets is critical for developing robust machine learning models that can accurately diagnose diseases from histopathological images. Inspired by recent advancements in multimodal learning, particularly the integration of visual and textual information, we focused on creating a comprehensive dataset specifically targeting keratinocyte cancers, including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).

This dataset consists of more than 2500 whole slide images (WSIs) from more than 1000 patients, meticulously collected and processed from two centres in Linz and Vienna to ensure diversity and representativeness. Each slide is accompanied by detailed metadata, including histopathological diagnoses, patient demographics, and diagnostic descriptions, enabling the development of machine learning models that can leverage both visual features and textual information.

By addressing the challenge of limited access to high-quality datasets in medical research, this project not only aims to facilitate the training and evaluation of cutting-edge AI models for skin cancer detection but also enhances the interpretability and reliability of these models in clinical settings. Our work lays the groundwork for future studies, fostering advancements in automated diagnosis and ultimately improving patient outcomes in dermatology.

P54

Altered Ca2+ homeostasis in keratinocytes of Darier disease patients might contribute to Th17-dominated skin infiltrate

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Darier disease (DD) is a rare genodermatosis that is caused by mutations in ATP2A2, encoding for sarco/endoplasmic reticulum calcium ATPase 2 (SERCA2). Patients present greasy papules and often malodorous plaques on seborrheic body areas, which significantly affect the quality of life of the individuals.

Traditionally, the effect of ATP2A2 mutations has been thought to be limited to keratinocytes. Recent research showed that DD patients have decreased numbers of peripheral B cells and increased numbers of lesional skin Th17 cells. However, whether this Th17-skewing is caused by keratinocyte-T cell interactions, by microbial colonization of lesional DD skin, or by the ATP2A2 mutation in the T cells per se, is not clear.

We established a primary, immortalized keratinocyte cell line heterozygously expressing a patient-specific ATP2A2 mutation. Using this in vitro model, we were able to show impaired differentiation behavior, altered Ca2+ homeostasis, and increased expression of IL23A, a Th17-promoting cytokine in SERCA2-mutated 2D keratinocyte cultures.

We conclude that ATP2A2 mutations might affect keratinocytes in a way that promotes the expression of Th17-priming cytokines thereby contributing to the Th17-dominated T cell infiltrate found in DD patients. However, this early evidence has to be confirmed by further 2D and 3D in vitro cell culture experiments.

P55

Transcriptional shifts at single-cell resolution in cutaneous sarcoidosis under mTOR inhibition

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Introduction: In a recent clinical trial, systemic inhibition of mTORC1 (mechanistic target of rapamycin complex 1) resulted in a long-lasting remission in a subset of patients with cutaneous sarcoidosis, a granulomatous disease. Skin and blood samples from various trial timepoints were analyzed using single-cell RNA sequencing, with spatial transcriptomics conducted on a subset of these samples. Our objective is to identify transcriptional changes in the tissue following mTOR inhibition and explore patterns associated with treatment response.

Methods: After quality control, high-quality single-cell transcriptomes from skin and blood were integrated using an scVI model, clustered, and annotated based on marker genes. Differential expression analysis between timepoints was performed using limma on pseudobulked counts. Monocle3 was used for trajectory inference. Cell subsets were mapped to the spatial slides using cell2location.

Results: Our transcriptomics data revealed all expected cell subsets, as well as distinct granuloma-specific populations. Significant transcriptional changes were observed in skin macrophages, helper T cells and fibroblasts after systemic treatment, predominantly in genes that are upregulated in sarcoidosis lesions compared to healthy tissue, resulting in a transcriptional profile resembling that of a normal state. These changes correlate with clinical response. We are now focusing on identifying the cell sub-populations most affected by the treatment, along with their functions and spatial location in the tissue. For instance, in macrophages, alterations in lipid metabolism, related to a divergence from the monocyte-derived macrophage differentiation trajectory, appear to generate granuloma-specific cells. The disappearance of this cell subset from the tissue is a key factor in the observed transcriptional changes.

Conclusions: Granulomatous skin undergoes significant transcriptional shifts following mTOR inhibitor treatment. Overall, the gene expression reverts to a state similar to healthy skin, driven by the loss of granuloma-specific macrophage, fibroblast and T cell populations within the tissue.

P56

Donor dependent variability of human CD34+ stemcell derived mast cells

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Background: Mast cells (MCs) are tissue-resident innate immune cells that are mostly present in connective and mucosal tissue, as well as in the tissue constituting the skin, lung, and gastrointestinal tract. For in vitro experiments large quantities of tissue is needed for MC isolation, or MC can be grown from blood-derived stem cells. The latter is easier accessible for research and results in higher yields of cells for experiments. Since repetitive experiments are usually needed for good scientific practice, we aimed in analyzing the variability of MC derived from different donors, to explore potential influencing factors for MC yield and receptor expression.

Methods: MC were generated from buffycoat via PBMC isolation and followed by CD34+ magnetic bead based selection. Cells were cultured for 4 weeks in serum free expansion medium (SFEM) as well as SCF and IL-3. Cells were counted for numbers every week during incubation and analyzed for the high affinity IgE receptor FceRIalpha expression via FACS staining. As functional degranulation assays Beta-hexosiminidase release was measured via enzymatic staining.

Results: CD34+ derived MC cultures resulted in cell numbers ranging from 1 225 000 cells per ml to 1 995 000 cells per ml after 2 weeks. Growth curves showed a broad variability. FACS analysis revealed between 85% and 91% percent MCs at week 2 and FceRIalpha expression on these cells between 55% and 80% percent.
Functional stimulation assays showed functionality of the cells when stimulated via IgE, anti-IgE and ionomycin.

Discussion: Human CD34+ stemcell derived MC show a large batch to batch variability. A higher number of batch analysis and its correlation with patient demographics is needed to draw meaningful conclusions that may allow a preselection of donors for successful MC culture.

P57

Deciphering the tissue-resident memory T cells – antigen presenting cell axis in skin and mucosa of persons living with HIV

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People living with HIV (PLWH) face a heightened risk of skin and mucosal diseases, such as Human Papilloma Virus (HPV)-related neoplasms, despite effective antiretroviral therapy (ART). The mechanisms driving this increased susceptibility among PLWH remain unclear. Tissue-resident memory T (TRM) cells in skin and mucosal tissues are essential for tissue immunity, protecting against infections and preventing carcinogenesis. Recent studies indicate dysregulation of protective CD4 TRM cells in the skin and mucosal tissue of ART-treated PLWH, though the underlying mechanisms remain elusive.

Since TRM cells require MHC presentation of cognate antigen for clonal expansion and activation, local dendritic cells as antigen-presenting cells (APCs) are likely critical to TRM cell homeostasis in the skin and mucosa. We analyzed single-cell RNA sequencing data from blood and skin of PLWH on long-term ART and HIV-negative controls. Differential gene expression analysis identified upregulation of inflammatory and inhibitory markers in TRM cells and APCs from PLWH, including SAT1, CD274, IL10RA, CCL2, CCL3, CCL4, IL1B and MMP15. Gene set enrichment analysis underscored pro-inflammatory and oncogenic gene modules in PLWH TRM cells and APCs, suggesting immune activation pathways that may cause chronic inflammation and disease vulnerability.

We also identified a notable correlation between APC and TRM cell gene signatures, suggesting intercellular crosstalk. LIANA, an interaction prediction analysis framework which aggregates multiple ligand-receptor resources demonstrated that MHC class II molecules on most APCs primarily interacted with the inhibitory receptor LAG-3 on CD4+ TRM cells in PLWH, an interaction remarkably lower in HIV-negative individuals, where MHC class II interactions on most APCs engaged the CD4 receptor.

APCs and TRM cells have similar transcriptional profiles and cellular morphologies in skin and mucosal tissues, suggesting that our findings may apply to mucosal tissues. We postulate that dysregulated APC-TRM interactions in PLWH impair cutaneous and mucosal TRM cell immunosurveillance, contributing to immune dysfunction.

P58

Aqp5 mutations in patients suffering from palmoplantar keratoderma (Bothnian type)

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Introduction: Aquaporins (Aqps) are structurally well conserved across all kingdoms of life and are divided into three main groups: (i) orthodox aquaporins, which conduct only water molecules, (ii) aquaglyceroporins, which channel water and other small uncharged solutes, and (iii) the less-studied unorthodox aquaporins. Water transport through these integral membrane channels occurs in a single file through the pores of each protomer, which form tetrameric units in cell membranes.
Here we focus on the functional consequences of mutations in orthodox Aqp5, which is widely expressed in glandular tissues and skin keratinocytes. These mutations cause hereditary PPKB, characterized by hyperkeratotic lesions on the surface of palms and soles.

Methods: 14 published Aqp5 mutations, were characterized by their position within the protein channels using PyMol and AlphaFold, and cloned them into yeast expression vectors. A yeast-based water assay exploited the expression of Aqp5 mutant proteins in a Saccharomyces cerevisiae aquaporin knockout strain.
Water permeability was measured using osmotically stressed lipid vesicles containing the reconstituted mutant proteins purified from Saccharomyces cerevisiae as YFP fusion proteins. The number of reconstituted channels was assessed by fluorescence correlation spectroscopy, allowing the calculation of unitary water channel conductance in vitro.

Results: While mutations situated within the water-conducting pore and on the protomer interfaces show effects in yeast-based and lipid vesicle-based methods, substitutions on the cytoplasmic surface of Aqp5 do not affect the conductance of the water channel.

Conclusion: In addition to transcellular water flux, which is regulated by pore-lining residues and tetramer stability, another mechanism must contribute to the phenotype of PPKB mutations. It seems reasonable to posit that Aqp5 plays a role in paracellular water flux through keratinocyte layers, given that all other mutations are located on the extracellular surface of Aqp5 and may serve as a platform for interacting proteins responsible for skin integrity.

P59

Cell selection mechanisms in X-linked development and disease

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The X chromosome contains more genetic material and genes than the Y, leading to an imbalance in gene expression between females (XX) and males (XY). In females, this imbalance is compensated through the epigenetic process of X-chromosome inactivation, during which one of the two X chromosomes is inactivated in every somatic cell. Which X will be inactive occurs randomly, and on average, tissues are assumed to be made up of cells with a 50:50 ratio of maternal: paternal X active (XCI ratio). However, the phenomenon of X skewing, wherein tissues are composed mainly of cells that have one specific X chromosome active, has been described in healthy adult tissues. Clinical studies have highlighted extreme levels of X-skewing in female carriers of X-linked diseases, suggesting X skewing and disease outcomes may be linked. Importantly, the developmental cellular dynamics and molecular mechanisms that underlie X-skewing have not been explored. Therefore, we aim to shed light on these fascinating unknowns.

We use the skin epidermis as a model system to investigate XCI ratios in healthy and diseased female mice across development. We hypothesise that X-skewing is a protective tissue-level mechanism that minimises the disruption of homeostasis caused by deleterious X-chromosome mutations. By combining reporter mice with mouse models of X-linked disease, we can correlate X-linked clonal dynamics to disease progression. Our preliminary data suggest unexpected relationships between the spatial position of X-mutant clones and disease phenotypes. We are also developing strategies that allow us to probe the mechanisms by which X-linked mutant cells may be sensed and eliminated from tissues to drive X-skewing. We hypothesise that a cell competition-like selection mechanism is likely to be involved in this process. Altogether, our work will illuminate exciting new insights on how cellular heterogeneity generated by X chromosome inactivation ratios impacts long-term tissue organisation and function.

P60

Mechanical determinants of cell growth and survival in embryonic skin formation

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Epithelial tissues, including the skin epidermis, shape our organs and provide a protective barrier. They contain heterogenous cell populations that differ in genetic and non-genetic attributes. Cell competition identifies and eliminates unfit cells, thereby maintaining tissue homeostasis. However, the molecular machinery behind fitness sensing and loser cell elimination remains poorly understood. I hypothesize that cellular mechanics, particularly cell-extracellular matrix (ECM) adhesions, play a pivotal role in fitness sensing. Despite evidence implicating mechanical factors such as cell density, crowding, and stiffness in cell elimination, the specific involvement of integrin-mediated cell-ECM adhesion sites remains unexplored. These adhesion sites play a pivotal role in regulating cell traction forces and cell contractility and are crucial to secure tissue homeostasis. Integrin receptors, which mediate cell-ECM binding, are key candidates in this process. I use the developing mouse epidermis as a model system to investigate the dynamics of cell-ECM adhesion remodeling during growth and fitness sensing. I hypothesize that variations in cell-ECM adhesion strength lead to mechanical competition among cells, affecting their fitness and ultimately determining their survival. To test this, I characterize composition and remodeling of ECM, integrins, and measure adhesive traction forces generated at cell-ECM adhesions. I aim to unravel how the balance of these mechanical forces during different developmental stages and during cell competition affects cell fitness and tissue homeostasis. My findings will not only address a fundamental question in development biology, but will also have far reaching implications for our understanding of how mechanical control of growth may go awry in disease.